EXHIBIT A

United States Court of Appeals for the Federal Circuit

2007-1400

ABBOTT LABORATORIES,

Plaintiff-Appellant,

and

ASTELLAS PHARMA, INC.,

Plaintiff-Appellant,

v.

SANDOZ, INC.,

Defendant-Appellee,

and

SANDOZ GMBH,

Defendant,

and

TEVA PHARMACEUTICALS USA, INC. and
TEVA PHARMACEUTICAL INDUSTRIES, LTD.,

Defendants-Appellees,

and

RANBAXY LABORATORIES, LTD. and RANBAXY, INC.,

Defendants,

and

PAR PHARMACEUTICAL COMPANIES, INC. and PAR PHARMACEUTICAL,

Defendants.

2007-1446

LUPIN LIMITED,

Plaintiff/Counterclaim Defendant-Appellee,

and

LUPIN PHARMACEUTICALS, INC.,

Counterclaim Defendant-Appellee,

v.

ABBOTT LABORATORIES,

Defendant/Counterclaimant-Appellant,

and

ASTELLAS PHARMA, INC.,

Defendant/Counterclaimant-Appellant.

James F. Hurst, Winston & Strawn LLP, of Chicago, Illinois, argued for all plaintiffs-appellants in 2007-1400 and defendants/counterclaimants-appellants in 2007-1446. With him on the briefs for Abbott Laboratories were Todd J. Ehlman, Kathleen B. Barry, and Ivan M. Poullaos, and Steffen N. Johnson, of Washington, DC. Of counsel on the brief for Abbott Laboratories were William F. Cavanaugh, Jr., Jeffrey I.D. Lewis, and Stuart E. Pollack, Patterson Belknap Webb & Tyler LLP, of New York, New York. Of counsel was John C. Knapp. On the briefs for Astellas Pharma, Inc., were Richard D. Kelly, Stephen G. Baxter and Frank J. West, Oblon, Spivak, McClelland, Maier & Neustadt, P.C., of Alexandria, Virginia.

Meredith Martin Addy, Brinks, Hofer, Gilson & Lione, of Chicago, Illinois, argued for defendant-appellee Sandoz, Inc. With her on the brief were Thomas J. Filarski, Mark H. Remus, C. Noel Kaman, and Laura A. Lydigsen. Of counsel was Rashad L. Morgan.

Thomas J. Meloro, Jr., Wilkie Farr & Gallagher LLP, of New York, New York, argued for defendants-appellees Teva Pharmaceuticals USA, Inc., et al. With him on the brief was Neal K. Feivelson. Of counsel were Michael W. Johnson and Alexander H. Swirnoff.

Deanne M. Mazzochi, Rakoczy Molino Mazzochi Siwik, LLP, of Chicago, Illinois, argued for plaintiff/counterclaim defendant-appellee Lupin Limited and counterclaim defendant-appellee, Lupin Pharmaceuticals, Inc. in 2007-1446. With her on the brief were William A. Rakoczy, Paul J. Molino, and Amy D. Brody.

Appealed from: United States District Court for the Northern District of Illinois (07-CV-1721)

Judge Wayne R. Andersen

Appealed from: United States District Court for the Eastern District of Virginia (3:06-CV-400)

Judge Robert E. Payne

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Defendant/Counterclaimant-Appellant.

Appeals from the United States District Court for the Northern District of Illinois in case no. 07-CV-1721, Judge Wayne R. Andersen and the United States District Court for the Eastern District of Virginia in case no 3:06-CV-400, Judge Robert E. Payne.

DECIDED: May 18, 2009

Before RADER, PLAGER, and BRYSON, Circuit Judges. MICHEL, Chief Judge, and RADER, BRYSON, GAJARSA, LINN, DYK, PROST, and MOORE, Circuit Judges, have joined Section III.A.2 of the opinion. Dissenting opinion as to Section III.A.2 filed by NEWMAN, Circuit Judge, in which MAYER and LOURIE, Circuit Judges, join. Dissenting opinion filed by LOURIE, Circuit Judge. SCHALL, Circuit Judge, did not participate as a member of the en banc court.

RADER, Circuit Judge.

In this case, the same patent, U.S. Patent No. 4,935,507 (the '507 patent), occasions litigation in both the United States District Court for the Eastern District of Virginia and the United States District Court for the Northern District of Illinois. The Virginia District Court granted the motion of Lupin Ltd. and Lupin Pharmaceuticals Inc. (collectively Lupin) for summary judgment of noninfringement. In the other case, the Illinois District Court denied a preliminary injunction to Abbott Laboratories, the exclusive licensee of the '507 patent, based on the claim construction from the Eastern District of Virginia.

Because the Eastern District of Virginia correctly construed the claims of the '507 patent and correctly discerned no genuine issues of material fact on literal infringement of claims 2-5 or infringement by equivalents of claims 1-5, this court affirms its partial summary judgment of noninfringement. Likewise, this court affirms the Northern District of Illinois' denial of Abbott's motion for a preliminary injunction, based in large part on the same correct claim construction.

I.

Abbott Laboratories, the exclusive licensee of the '507 patent, markets crystalline cefdinir according to the '507 patent under the trade name Omnicef. The Virginia case arose when Lupin sought a declaratory judgment of noninfringement against Abbott Laboratories and Astellas Pharma Inc., the owner of the '507 patent (collectively Abbott). The Food and Drug Administration had previously approved Lupin's Abbreviated New Drug Application (ANDA) to market a generic version of Omnicef. Lupin's generic product contains almost exclusively the Crystal B form of crystalline

cefdinir (cefdinir monohydrate), whereas Abbott's Omnicef product contains the Crystal A form of crystalline cefdinir (cefdinir anhydrate). Further, Lupin makes its products with processes other than those claimed in the '507 patent. For these reasons, Lupin brought the Virginia action to clarify that its proposed product would not infringe a valid patent. Abbott counterclaimed for infringement. The Eastern District of Virginia construed the claims, Lupin Ltd. v. Abbott Laboratories, 484 F. Supp. 2d 448 (E.D. Va. 2007) (Lupin CC Order), and ultimately granted-in-part Lupin's motion for summary judgment of noninfringement, as to both literal and equivalent infringement for claims 2-5 and as to equivalent infringement for claim 1, Lupin Ltd. v. Abbott Labs., 491 F. Supp. 2d 563 (E.D. Va. 2007) (Lupin SJ Order). The parties stipulated to the dismissal without prejudice of the remaining claims (invalidity) and counterclaims (literal infringement of claim 1).

In the Illinois action, Abbott sued Sandoz, Inc. and Sandoz GmbH (collectively Sandoz), Teva Pharmaceuticals USA, Inc. and Teva Pharmaceuticals Industries, Ltd. (collectively Teva), Ranbaxy Laboratories, Ltd., Ranbaxy, Inc., Par Pharmaceutical Companies, Inc., and Par Pharmaceutical (all defendants, collectively, Sandoz and Teva) for infringement of the '507 patent. Like Lupin, Sandoz and Teva had previously filed ANDAs, seeking to market generic versions of Omnicef. Abbott sought a preliminary injunction in the Illinois case. For purposes of that motion, the parties agreed to adopt the Eastern District of Virginia's claim construction from the Lupin case. Abbott Labs. v. Sandoz, Inc., 486 F. Supp. 2d 767 (N.D. Ill. 2007) (Sandoz PI Order). Despite this agreement, the parties to the Sandoz case disagreed as to how to interpret some of the Eastern District of Virginia's constructions, necessitating some clarification

by the Northern District of Illinois. 486 F. Supp. 2d at 770-71 (disputing "Crystal A," "peaks," and "about," and seeking construction of "powder X-ray diffraction pattern," which the Eastern District of Virginia had not defined). Ultimately, the Northern District of Illinois, based on the claim construction from Virginia, denied the preliminary injunction.

Both cases arrived at this court on appeal. This court heard the cases together and decides them together with this decision.

II.

The '507 patent has five claims, all of which Abbott asserts against Lupin as well as Sandoz and Teva. Claim 1 claims crystalline cefdinir, using its chemical name, and defining its unique characteristics with powder X-ray diffraction (PXRD) angle peaks:

> 1. Crystalline 7-[2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-vinyl-3-cephem.-4-carboxylic acid (syn isomer) which shows the peaks at the diffraction angles shown in the following table in its powder X-ray diffraction pattern:

diffraction angle (°)
about 14.7°
about 17.8°
about 21.5°
about 22.0°
about 23.4°
about 24.5°
about 28.1°

'507 patent, col.16 ll.13-27. In contrast, claims 2-5 claim crystalline cefdinir, without any PXRD peak limitations, but with descriptions of processes used to obtain the crystalline cefdinir. Claims 2 and 5 are independent:

> 2. Crystalline 7-[2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-vinyl-3-cephem-4-carboxylic acid (syn isomer) which is obtainable by

> acidifying a solution containing 7-[2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-vinyl-3-cephem-4-carboxylic acid (syn isomer) at room temperature or under warming.
>
> 5. Crystalline 7-[2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-vinyl-3-cephem-4-carboxylic acid (syn isomer) which is obtainable by dissolving 7-[2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-vinyl-3-cephem-4-carboxylic acid (syn isomer) in an alcohol, continuing to stir the solution slowly under warming, then cooling the solution to room temperature and allowing the solution to stand.

Id. at col.16 ll.29-34, 43-50.

These claims use PXRD as a way to claim the structure and characteristics of the unique crystalline form. PXRD is a method for identifying and distinguishing different crystalline compounds. The method beams X-rays toward a powdered chemical. The method then measures the ways the rays reflect or bend upon contact with the chemical. The diffraction angles and intensities vary with the type and purity of the test compound. A graph then plots the diffraction angle on one axis and the intensity on another. These graphs yield a unique "fingerprint" for each crystalline form of a chemical. A more sensitive form of X-ray diffraction is single crystal X-ray diffraction (SCXRD). As this name suggests, this method uses only a single crystal as a sample. SCXRD does not detect intensity, but produces a more precise diffraction angle measurement.

The '507 patent was not the first cefdinir patent. Rather, Astellas' prior art U.S. Patent No. 4,559,334 (the '334 patent) describes the discovery of cefdinir as a compound demonstrating high antimicrobial activity. '334 patent, col.11 ll.18-24. The '334 patent expired on May 6, 2007.

The '507 patent claims priority to Japanese Patent Application No. 62-206199 (the JP '199 application), which claimed two crystalline forms of cefdinir, "Crystal A" and

"Crystal B." The JP '199 application claimed Crystals A and B very specifically, defining Crystal A by three infrared (IR)-absorption wavelengths and sixteen PXRD angles and intensities. In contrast, Crystal B featured five IR-absorption wavelengths and twenty-one PXRD angles/intensities.

Despite using the JP '199 application for priority, the '507 patent's specification differs significantly. Specifically, Abbott (actually Fujisawa Pharmaceutical Co., Ltd., Astellas' predecessor in interest) jettisoned the Crystal B disclosure found in the JP '199 application and crafted broader claims in its prosecution of the '507 patent. Because the JP '199 applications defines Crystal A and Crystal B physiochemically rather than structurally, the forms actually represent subgenuses of crystalline cefdinir. Thus Crystals A and B comprise crystalline forms of varying structures, which in the context of this case means varying levels of hydration.

The Eastern District of Virginia construed the claim terms "crystalline," "shows," "peaks," and "about" as follows:

> 1) "crystalline" means "Crystal A as outlined in the specification";
>
> 2) "shows" requires the display of a powder X-ray diffraction pattern which demonstrates the existence of the relevant peaks to a scientifically acceptable degree of certainty either visually or by other appropriate means of data display;
>
> 3) "peaks" is the plural of "peak;" a "peak exists at a powder X-ray diffraction angle that corresponds to an intensity measurement greater than measurements attributable to "noise" if that angle is immediately preceded by and followed by powder X-ray diffraction angle with a lower intensity measurement; "noise" refers to those portions of a PXRD pattern produced by intrinsic measurement error, and which cannot be associated with a scientifically significant quantity of the material which is the subject of the PXRD test;
>
> 4) "about" encompasses measurement errors inherently associated with powder X-ray diffraction testing.

Lupin CC Order, 484 F. Supp. 2d at 459, 466. The Eastern District of Virginia also concluded that claims 2-5 were product-by-process claims. Id. Later the district court concluded that the process terms of claims 2-5, indicated by the phrase "obtainable by," limit the claims to the specified processes and process steps. In reaching that conclusion, the trial court followed this court's opinion in Atlantic Thermoplastics Co. v. Faytex Corp., 970 F.2d 834 (Fed. Cir. 1992). Lupin SJ Order, 491 F. Supp. 2d at 567-68; Lupin Ltd. v. Abbott Labs., No. 3:06-CV-400 (E.D. Va. May 10, 2007) (Lupin PbyP Order). In the Lupin appeal, Abbott challenges only the Eastern District of Virginia's constructions of "crystalline" and "obtainable by."

III.

Evaluation of a summary judgment of noninfringement requires two steps: claim construction, which this court reviews without deference, Cybor Corp. v. FAS Technologies, Inc., 138 F.3d 1448, 1451 (Fed. Cir. 1998) (en banc), and comparison of the properly construed claims to the accused product, process, or composition of matter, which in the context of summary judgment also occurs without deference, see Ormco Corp. v. Align Technologies, Inc., 498 F.3d 1307, 1312 (Fed. Cir. 2007). Although infringement by equivalency is a question of fact, this court may affirm summary judgment "where no reasonable fact finder could find equivalence." Sage Prods., Inc. v. Devon Indus., Inc., 126 F.3d 1420, 1423 (Fed. Cir. 1997) (citing Warner-Jenkinson Co. v. Hilton Davis Chemical Co., 520 U.S. 17, 39 n.8 (1997).

A. Claim Construction

Because the claims define the patent right, see Innova/Pure Water, Inc. v. Safari Water Filtration Systems, Inc., 381 F.3d 1111, 1115 (Fed. Cir. 2004), naturally "the

claims themselves provide substantial guidance as to the meaning of particular claim terms." Phillips v. AWH Corp., 415 F.3d 1303, 1314 (Fed. Cir. 2005) (en banc). But the claims "must be read in view of the specification, of which they are a part." Markman v. Westview Instruments, Inc., 52 F.3d 967, 979 (Fed. Cir. 1995) (en banc), aff'd, 517 U.S. 370 (1996). A patent's specification provides necessary context for understanding the claims, and "is always highly relevant to the claim construction analysis." Phillips, 415 F.3d at 1315 (quoting Vitronics Corp. v. Conceptronic, Inc., 90 F.3d 1576, 1582 (Fed. Cir. 1996)). While equally true in a general sense, sometimes the specification offers practically incontrovertible directions about claim meaning. For example, inventors may act as their own lexicographers and give a specialized definition of claim terms. See id. at 1316. Likewise, inventors and applicants may intentionally disclaim, or disavow, subject matter that would otherwise fall within the scope of the claim. See id.

When consulting the specification to clarify the meaning of claim terms, courts must take care not to import limitations into the claims from the specification. This court has recognized the "fine line between" the encouraged and the prohibited use of the specification. Comark Commc'ns, Inc. v. Harris Corp., 156 F.3d 1182, 1186 (Fed. Cir. 1998). When the specification describes a single embodiment to enable the invention, this court will not limit broader claim language to that single application "unless the patentee has demonstrated a clear intention to limit the claim scope using 'words or expressions of manifest exclusion or restriction.'" Liebel-Flarsheim Co. v. Medrad, Inc., 358 F.3d 898, 906 (Fed. Cir. 2004) (quoting Teleflex, Inc. v. Ficosa N. Am. Corp., 299 F.3d 1313, 1327 (Fed. Cir. 2002)). By the same token, the claims cannot "enlarge what is patented beyond what the inventor has described as the invention." Biogen, Inc. v.

Berlex Labs., Inc., 318 F.3d 1132, 1140 (Fed. Cir. 2003) (quoting Netword, LLC v. Central Corp., 242 F.3d 1347, 1352 (Fed. Cir. 2001)). Thus this court may reach a narrower construction, limited to the embodiment(s) disclosed in the specification, when the claims themselves, the specification, or the prosecution history clearly indicate that the invention encompasses no more than that confined structure or method. See Liebel-Flarsheim, 358 F.3d at 908.

Along with the specification, the prosecution history is "intrinsic evidence" of the meaning of the claims, because it "provides evidence of how the [United States Patent & Trademark Office (PTO)] and the inventor understood the patent." Phillips, 415 F.3d at 1317. Although often producing ambiguities occasioned by ongoing negotiations between the inventor and the PTO, "the prosecution history can often inform the meaning of the claim language by demonstrating how the inventor understood the invention and whether the inventor limited the invention in the course of prosecution, making the claim scope narrower than it would otherwise be." Id. "[C]lear and unmistakable" statements during prosecution may also disavow claim scope. Computer Docking Station Corp. v. Dell, Inc., 519 F.3d 1366, 1374 (Fed. Cir. 2008) (quoting Purdue Pharma L.P. v. Endo Pharms., Inc., 438 F.3d 1123, 1136 (Fed. Cir. 2006)). Again owing in part to the inherent ambiguities of prosecution history, the doctrine of prosecution disclaimer only applies to unambiguous disavowals. See id. at 1375.

1. "crystalline"

The Eastern District of Virginia's construction of "crystalline" in claims 1-5 as "Crystal A" included the important caveat "as outlined in the specification." Lupin CC Order, 484 F. Supp. 2d at 459. Although the Eastern District noted the parties agreed that "crystalline" ordinarily means exhibiting "uniformly arranged molecules or atoms," id. at 454, the court relied on the language of the claims themselves, the specification, and the prosecution history to arrive at the more specific meaning recited in the specification.

The '507 specification states that "Crystal A of the compound (I) [cefdinir] shows its distinguishing peaks" at the seven particular PXRD angles enumerated in claim 1. '507 patent col.1 ll.51-62. Indeed, the phrase "Crystal A of the compound (I)" appears throughout the written description, and the patent offers the following definition: "any crystal of the compound (I) which shows substantially the same diffraction pattern [as in the table in col.1/claim 1] is identified as Crystal A of the compound (I)." Id. at col.1 l.67-col.2 l.2. As the Eastern District correctly concluded:

> Had Astellas intended, in the chart found in column 1, to distinguish Crystal A from other forms of crystalline cefdinir that also fall within the scope of claim 1, it would have listed, at a minimum, an eighth peak associated only with Crystal A. However, by listing in column 1 only the same seven 'distinguishing' peaks featured in Claim 1, Astellas confirmed that Crystal A was synonymous with the invention listed in Claim 1.

Lupin CC Order, 484 F. Supp. 2d at 456-57. The problem, within the confines of claim 1, is that defining "crystalline" as "Crystal A," where "Crystal A" incorporates the seven PXRD peak limitations, arguably renders the remainder of that claim redundant. To distinguish the invention, however, the specification refers several times to "Crystal A of the compound (I) of the present invention," see, e.g., '507 patent, col.2 ll.15-17, and offers no suggestion that the recited processes could produce non-Crystal A

compounds, even though other types of cefdinir crystals, namely Crystal B, were known in the art. As noted earlier, the Crystal B formulation actually appears in the parent JP '199 application. Thus, Abbott knew exactly how to describe and claim Crystal B compounds. Knowing of Crystal B, however, Abbott chose to claim only the A form in the '507 patent. Thus, the trial court properly limited the term "crystalline" to "Crystal A." The trial court's definition correctly identifies claim 1's literal scope.

Unlike claim 1, claims 2-5 do not recite the seven PXRD peaks expressly associated with Crystal A in the '507 specification. Nonetheless, the Eastern District of Virginia limited "crystalline" to "Crystal A" in these claims as well, The trial court gave two reasons for this limitation. First, "[t]he process steps detailed in those claims [claims 2-5] correspond with the processes for making Crystal A disclosed in the specification under the heading 'The Process For Preparing Crystal A of The Compound (I).'" Id. at 457 (quoting '507 patent, col.2 ll.13-14). Second, the parent JP '199 application recited these steps "to distinguish between preparations of Crystal A and Crystal B." Id. (citing JP '199 application, col.6 ll.1-25).

In limiting "crystalline" to "Crystal A" in claims 1-5, the Eastern District of Virginia did not improperly import the preferred embodiment into the claims. Initially, Crystal A is the only embodiment described in the specification. As discussed above, the specification's recitation of Crystal A as its sole embodiment does not alone justify the trial court's limitation of claim scope to that single disclosed embodiment. See Liebel-Flarsheim, 358 F.3d at 906 ("[T]his court has expressly rejected the contention that if a patent describes only a single embodiment, the claims of the patent must be construed as being limited to that embodiment."). In this case, however, the rest of the intrinsic

evidence, including the prosecution history and the priority JP '199 application, evince a clear intention to limit the '507 patent to Crystal A as defined by the seven PXRD peaks in the specification and in claim 1.

Initially, the Eastern District of Virginia properly considered the JP '199 application as relevant objective evidence of the inventor's knowledge at the filing of the '507 patent. While statements made during prosecution of a foreign counterpart to a U.S. patent application have a narrow application to U.S. claim construction, Pfizer Inc. v. Ranbaxy Labs. Ltd., 457 F.3d 1284, 1290 (Fed. Cir. 2006), in this case the JP '199 application is part of the prosecution history of the '507 patent itself. Indeed the '507 patent claims priority from the JP '199 application. Furthermore, the trial court did not rely on attorney argument or amendments during a foreign prosecution as in Pfizer, but consulted only the contents of the foreign priority application. The JP '199 application strongly suggests that the '507 patent intentionally excluded Crystal B compounds. As discussed above, the JP '199 application establishes unequivocally that Abbott knew and could describe both Crystal A and Crystal B. Abbott could have retained the disclosure of Crystal B to support the broader claims of the '507 patent, but instead disclosed and claimed A alone.

Furthermore, the prosecution history of the '507 patent shows a clear and intentional disavowal of claim scope beyond Crystal A. Co-inventor Takao Takaya, who prepared samples according to Examples 14 and 16 of the prior art '334 patent and a sample of "Crystal A of the present application," offered a declaration that Crystal A was more stable than the prior art samples from the '334 patent. An analytical chemist, Yoshihiko Okamato, corroborated this evidence. J. A. 501-04. Beyond these

declarations, the applicant specifically limited the invention to Crystal A: "the method of preparation of the crystalline form of the presently claimed compounds is not considered the heart of the present invention. The crystalline form of the compound represents the inventive concept hereof, and it is clear that [the '334 patent] does not anticipate or suggest said crystalline form." J. A. 511 (Response to Office Action of May 11, 1989, received October 27, 1989, at 6).

Given the exclusive focus on Crystal A in the specification as well as the prosecution history of the '507 patent, the Eastern District of Virginia properly limited "crystalline" in claims 1-5 to "Crystal A."

2. proper interpretation of product-by process claims[1]

This court addresses Part III.A.2 of this opinion en banc, which addresses the proper interpretation of product-by-process claims in determining infringement.

Claims 2-5 of the '507 patent begin by reciting a product, crystalline cefdinir, and then recite a series of steps by which this product is "obtainable." The Eastern District of Virginia correctly categorized claims 2-5 as product-by-process claims. On appeal, Abbott argues that the Eastern District erred in construing the process steps of claims 2-5 under the rule in Atlantic Thermoplastics, 970 F.2d at 846-47, that "process terms in product-by-process claims serve as limitations in determining infringement," rather than in accordance with Scripps Clinic & Research Foundation v. Genentech, Inc., 927 F.2d 1565, 1583 (Fed. Cir. 1991) ("[T]he correct reading of product-by-process claims is that

[1] This court, sua sponte, took en banc Section III.A.2 before issuing a panel opinion. The following judges join this section of the opinion: Chief Judge Michel and Judges Rader, Bryson, Gajarsa, Linn, Dyk, Prost, and Moore. Judges Newman and Lourie dissent in separate opinions. Judges Mayer and Lourie join in Judge Newman's dissent. Judge Schall did not participate as a member of the en banc court.

they are not limited to product prepared by the process set forth in the claims."). This court takes this opportunity to clarify en banc the scope of product-by-process claims by adopting the rule in Atlantic Thermoplastics.

In Atlantic Thermoplastics, this court considered the scope of product-by-process claim 26 in the patent at issue: "[t]he molded innersole produced by the method of claim 1." 970 F.2d at 836. The patentee urged that competing, indistinguishable innersoles made by a different method nonetheless infringed claim 26. Id. at 838. This court rejected the patentee's position. This court in Atlantic Thermoplastics construed product-by-process claims as limited by the process. Id. at 846-7.

This rule finds extensive support in Supreme Court opinions that have addressed the proper reading of product-by-process claims. See Smith v. Goodyear Dental Vulcanite Co., 93 U.S. 486, 493 (1877) ("The process detailed is thereby made as much a part of the invention as are the materials of which the product is composed."); Goodyear Dental Vulcanite Co. v. Davis, 102 U.S. 222, 224 (1880) ("[T]o constitute infringement of the patent, both the material of which the dental plate is made . . . and the process of constructing the plate . . . must be employed."); Merrill v. Yeomans, 94 U.S. 568 (1877); Cochrane v. Badische Anilin & Soda Fabrik, 111 U.S. 293 (1884) (BASF); The Wood-Paper Patent, 90 U.S. 566, 596 (1874); Plummer v. Sargent, 120 U.S. 442 (1887); Gen. Elec. Co. v. Wabash Appliance Corp., 304 U.S. 364 (1938); see also Atl. Thermoplastics, 970 F.2d at 839-42 (discussing each of these cases). In these cases, the Supreme Court consistently noted that process terms that define the product in a product-by-process claim serve as enforceable limitations. In addition, the binding case law of this court's predecessor courts, the United States Court of Customs

and Patent Appeals (see In re Hughes, 496 F.2d 1216, 1219 (CCPA 1974) (acknowledging that "true product claims" are "broader" in scope than product-by-process claims)), and the United States Court of Claims (see Tri-Wall Containers v. United States, 408 F.2d 748, 751 (Ct. Cl. 1969)), followed the same rule.

This court's sister circuits also followed the general rule that the defining process terms limit product-by-process claims. See, e.g., Hide-Ite Leather v. Fiber Prods., 226 F. 34, 36 (1st Cir. 1915) ("It is also a well-recognized rule that, although a product has definite characteristics by which it may be identified apart from the process, still, if in a claim for the product it is not so described, but is set forth in the terms of the process, nothing can be held to infringe the claim which is not made by the process."); Paeco, Inc. v. Applied Moldings, Inc., 562 F.2d 870, 876 (3d Cir. 1977) ("A patent granted on a product claim describing one process grants no monopoly as to identical products manufactured by a different process."). Indeed, this court itself had articulated that rule: "For this reason, even though product-by-process claims are limited by and defined by the process, determination of patentability is based on the product itself." In re Thorpe, 777 F.2d 695, 697 (Fed. Cir. 1985) (emphasis added).

The Supreme Court has long emphasized the limiting requirement of process steps in product-by-process claims. In BASF, the Court considered a patent relating to artificial alizarine. Specifically, the patent claimed "[a]rtificial alizarine, produced from anthracine or its derivatives by either of the methods herein described, or by any other method which will produce a like result." 111 U.S. at 296 (quoting U.S. Patent Reissue No. RE 4,321). In turn, the specification generally described a method for making artificial alizarine involving anthracine or its derivatives. Alizarine had been in use for

thousands of years as a red textile dye, traditionally extracted from madder root. Pure alizarine has the chemical formula $C_{14}H_8O_4$, but "artificial alizarines" available in the market at the time of the litigation varied from almost completely pure alizarine, to combinations of alizarine and anthrapurpurine, to pure purpurine containing no alizarine whatsoever. Id. at 309-10. The defendant's product contained approximately sixty percent anthrapurpurine. Thus both alizarine and artificial alizarines were known in the prior art. The Supreme Court clearly articulated some of the scope and validity problems that arise when process limitations of product-by-process claims are ignored:

> [The defendant's product] is claimed by the plaintiff to be the artificial alizarine described in No. 4,321, and to be physically, chemically, and in coloring properties similar to that. But what that is is not defined in No. 4,321, except that it is the product of the process described in No. 4,321. Therefore, unless it is shown that the process of No. 4,321 was followed to produce the defendant's article, or unless it is shown that that article could not be produced by any other process, the defendant's article cannot be identified as the product of the process of No. 4,321. Nothing of the kind is shown.
>
> * * *
>
> If the words of the claim are to be construed to cover all artificial alizarine, whatever its ingredients, produced from anthracine or its derivatives by methods invented since Graebe and Liebermann invented the bromine process, we then have a patent for a product or composition of matter which gives no information as to how it is to be identified. Every patent for a product or composition of matter must identify it so that it can be recognized aside from the description of the process for making it, or else nothing can be held to infringe the patent which is not made by that process.

Id. at 310 (emphasis added).

After BASF, the Supreme Court continued to emphasize the importance of process steps in evaluating the infringement of product-by-process claims. See, e.g., Plummer, 120 U.S. at 448 ("[W]hatever likeness that may appear between the product of the process described in the patent and the article made by the defendants, their

identity is not established unless it is shown that they are made by the same process."); Gen. Elec. Co., 304 U.S. at 373 ("[A] patentee who does not distinguish his product from what is old except by reference, express or constructive, to the process by which he produced it, cannot secure a monopoly on the product by whatever means produced." (footnote omitted)).

Thus, based on Supreme Court precedent and the treatment of product-by-process claims throughout the years by the PTO and other binding court decisions, this court now restates that "process terms in product-by-process claims serve as limitations in determining infringement." Atl. Thermoplastics, 970 F.2d at 846-47. As noted earlier, this holding follows this court's clear statement in In re Thorpe that "product by process claims are limited by and defined by the process." 777 F.2d at 697.

More recently, the Supreme Court has reiterated the broad principle that "[e]ach element contained in a patent claim is deemed material to defining the scope of the patented invention." Warner-Jenkinson, 520 U.S. at 19. Although Warner-Jenkinson specifically addressed the doctrine of equivalents, this rule applies to claim construction overall. As applied to product-by-process claims, Warner-Jenkinson thus reinforces the basic rule that the process terms limit product-by-process claims. To the extent that Scripps Clinic is inconsistent with this rule, this court hereby expressly overrules Scripps Clinic.

The dissenting opinions lament the loss of a "right" that has never existed in practice or precedent – the right to assert a product-by-process claim against a defendant who does not practice the express limitations of the claim. This court's en banc decision in no way abridges an inventor's right to stake claims in product-by-

process terms. Instead this decision merely restates the rule that the defining limitations of a claim – in this case process terms – are also the terms that show infringement.

Thus this court does not question at all whether product-by-process claims are legitimate as a matter of form. The legitimacy of this claim form was indeed a relevant issue in the nineteenth century when Ex parte Painter, 1891 C.D. 200, 200-01 (Comm'r Pat. 1891), and some later cases were before the Commissioner of Patents. However, this court need not address that settled issue. The issue here is only whether such a claim is infringed by products made by processes other than the one claimed. This court holds that it is not.

The jurisprudence of the Court of Customs and Patent Appeals – a court with virtually no jurisdiction to address infringement litigation – can shed little light on the enforcement of the only claim limitations that an applicant chooses to define the invention. Indeed, this court's venerable predecessor expressed its ambivalence towards the relevant infringement analysis:

> The policy of the Patent Office in permitting product-by-process type claims to define a patentable product, where necessary, has developed with full cognizance of the fact that in infringement suits some courts have construed such claims as covering only a product made by the particular process set forth in the claim and not to the product per se.

In re Bridgeford, 357 F.2d 679, 683 n.5 (CCPA 1966). The reference to "some courts" in this prior citation, as this court notes en banc, includes the United States Supreme Court and every circuit court to consider the question, including this circuit. See also Jon S. Saxe & Julian S. Levitt, Product-by-Process Claims and Their Current Status in Chemical Patent Office Practice, 42 J. Pat. Off. Soc'y 528, 530 (1960) ("[P]roduct-by-

process claims have met with a most strict interpretation in the courts in infringement proceedings . . . . [T]he courts uniformly hold that only a product produced by the claim-designated process may be held to infringe the claim.") (citing Gen. Elec. Co., 304 U.S. 364 and BASF, 111 U.S. at 310).

Product-by-process claims, especially for those rare situations when products were difficult or impossible to describe, historically presented a concern that the Patent Office might deny all product protection to such claims. See In re Butler, 17 C.C.P.A. 810, 813 (CCPA 1930) ("Process claims are valuable, and appellant thinks he is entitled to them; but it is submitted that he should not be limited to control of the process when the article which that process produces is new and useful."). In the modern context, however, if an inventor invents a product whose structure is either not fully known or too complex to analyze (the subject of this case – a product defined by sophisticated PXRD technology – suggests that these concerns may no longer in reality exist), this court clarifies that the inventor is absolutely free to use process steps to define this product. The patent will issue subject to the ordinary requirements of patentability. The inventor will not be denied protection. Because the inventor chose to claim the product in terms of its process, however, that definition also governs the enforcement of the bounds of the patent right. This court cannot simply ignore as verbiage the only definition supplied by the inventor.

This court's rule regarding the proper treatment of product-by-process claims in infringement litigation carries its own simple logic. Assume a hypothetical chemical compound defined by process terms. The inventor declines to state any structures or characteristics of this compound. The inventor of this compound obtains a product-by-

process claim: "Compound X, obtained by process Y." Enforcing this claim without reference to its defining terms would mean that an alleged infringer who produces compound X by process Z is still liable for infringement. But how would the courts ascertain that the alleged infringer's compound is really the same as the patented compound? After all, the patent holder has just informed the public and claimed the new product solely in terms of a single process. Furthermore, what analytical tools can confirm that the alleged infringer's compound is in fact infringing, other than a comparison of the claimed and accused infringing processes? If the basis of infringement is not the similarity of process, it can only be similarity of structure or characteristics, which the inventor has not disclosed. Why also would the courts deny others the right to freely practice process Z that may produce a better product in a better way?

In sum, it is both unnecessary and logically unsound to create a rule that the process limitations of a product-by-process claim should not be enforced in some exceptional instance when the structure of the claimed product is unknown and the product can be defined only by reference to a process by which it can be made. Such a rule would expand the protection of the patent beyond the subject matter that the inventor has "particularly point[ed] out and distinctly claim[ed]" as his invention, 35 U.S.C. § 112 ¶ 6.

Thus, the Eastern District of Virginia correctly applied the rule that the recited process steps limit the product-by-process claims 2-5 for any infringement analysis.

3. "obtainable by"

In this case, Abbott's plain language argument, that "obtainable by" introduces an optional process, even if "obtained by" would introduce limiting process steps, is also unavailing. The BASF case, an analogous situation to this case, controls. As noted above, the Supreme Court in BASF considered the following claim language: "Artificial alizarine, produced from anthracine or its derivatives by either of the methods herein described, or by any other method which will produce a like result." 111 U.S. at 296 (emphasis added). The patentee argued that even though the defendant did not make artificial alizarine by "either of the methods herein described," the claim should capture the product because of the "or by another method" language. Id. at 309. The Supreme Court refused to attach importance to those expansive words: "No. 4,321 furnishes no test by which to identify the product it covers, except that such product is to be the result of the process it describes." Id. at 305. Abbott's claims 2-5, like those in BASF and like product-by-process claims in general, do not furnish any test by which to identify the cefdinir crystals except that they are the result of their respectively claimed processes. As per BASF, Abbott's claim cannot capture a product obtained by or obtainable by processes other than those explicitly recited in the claims.

If this court were to strip the process elements from the claims, as Abbott would urge, for infringement purposes, there would then be nothing to differentiate independent claim 2 from independent claim 5. After all, if those claims are not bound by the process terms but only "define" the basic cefdinir compound, then each of the claims recite the same thing, over and over again. Though Abbott argues that it merely intends to give meaning to the word "obtainable," it instead seeks to have this court

render meaningless the explicit process limitations that the applicant chose to define its invention.

The intrinsic evidence in this case further rebuts Abbott's contention that its claims are not limited to those products actually obtained by the processes recited. In column 2 of the '507 patent, under the title heading "The Process for Preparing Crystal A of the Compound (I)," the patentee used specific language to describe the very two processes that are mirrored in claims 2 and 5. '507 patent col.2 ll.13-51. This language is not open-ended, nor does it constitute a mere description of the product by reference to the manner in which it can be made, as Abbott argues. By drafting claims 2 and 5 to incorporate these specific processes, Abbott made a conscious choice to place process requirements on its claimed product. If Abbott had wanted to obtain broader coverage for crystalline cefdinir devoid of any process limitations, as it seeks to do here, it could have simply done so (if indeed, as it argues, it is really the product that is the heart of the invention, not the process). But it did not. The crystals of claims 2 and 5 are simply not identifiable other than by the processes disclosed in column 2. This court must enforce the ways and terms that a party chooses to define its invention.

The prosecution history also does not support Abbott's contention that "obtainable by" offers merely an optional set of definitional process conditions. During prosecution, Abbott faced obviousness rejections based on application claims 6-9, which were process claims that mirrored the very process limitations of issued claims 2-5. The PTO refused to issue the claims until one set of duplicates was cancelled. Abbott's action in cancelling claims 6-9 demonstrates its acquiescence to the PTO's view that the process elements of claims 2-5 are critical parts of those claims. In

addition, in a response to the PTO's office action, Abbott chose to differentiate a cited § 103 reference, Takaya, on the basis that Abbott's claimed processes are different. For these reasons, the applicant's statement in the file wrapper that "the method of preparation . . . is not considered the heart of the present invention" should not be afforded undue gravitas. The process limitations cannot be haphazardly jettisoned for an infringement analysis when they were so important in the patentability analysis.

In sum, a patentee's use of the word "obtainable" rather than "obtained by" cannot give it a free pass to escape the ambit of the product-by-process claiming doctrine. Claims that include such ambiguous language should be viewed extremely narrowly. If this court does not require, as a precondition for infringement, that an accused infringer actually use a recited process, simply because of the patentee's choice of the probabilistic suffix "able," the very recitation of that process becomes redundant. This would widen the scope of the patentee's claims beyond that which is actually invented—a windfall to the inventor at the expense of future innovation and proper notice to the public of the scope of the claimed invention. For all the above reasons, the Eastern District of Virginia correctly construed the process limitations beginning with "obtainable by" in claims 2-5 as limiting the asserted claims to products made by those process steps.

B. Summary Judgment

In the Lupin case, the Eastern District of Virginia granted summary judgment of noninfringement of claims 2-5, both literal and by equivalents, and of claim 1 by equivalents. Lupin SJ Order. Literal infringement of claim 1, i.e., whether Lupin's generic cefdinir product contains any Crystal A, is therefore not a live issue on appeal.

As for claims 2-5, the Eastern District noted that "Abbott and Astellas have conceded that literal infringement of Claims 2-5 cannot be established if the product-by-process analysis is performed pursuant to Atlantic Thermoplastics," given that "Abbott and Astellas have presented no evidence that Lupin is practicing the process steps set forth in Claims 2-5." Lupin SJ Order, 491 F. Supp. 2d at 568. Because the Eastern District correctly applied the rule from Atlantic Thermoplastics and likewise properly construed the limiting process terms in claims 2-5, only infringement by equivalents of claims 1-5 remains before this court.

Infringement analysis under the doctrine of equivalents proceeds element-by-element; a generalized showing of equivalency between the claim as a whole and the allegedly infringing product or process is not sufficient to show infringement. See Warner-Jenkinson, 520 U.S. at 29 ("the doctrine of equivalents must be applied to individual elements of the claim, not to the invention as a whole"). The primary test for equivalency is the "function-way-result" or "triple identity" test, whereby the patentee may show an equivalent when the accused product or process performs substantially the same function, in substantially the same way, to achieve substantially the same result, as disclosed in the claim. Graver Tank & Mfg. Co. v. Linde Air Prods. Co., 339 U.S. 605, 608 (1950). But, because "[d]ifferent linguistic frameworks may be more suitable to different cases," Warner-Jenkinson, 520 U.S. at 40, the function-way-result test is not the only test for equivalency. Equivalency may also be proven where the differences between the invention as claimed and the accused product or process are insubstantial. Hilton Davis Chem. Co. v. Warner-Jenkinson Co., 62 F.3d 1512, 1517-18 (Fed. Cir. 1995) (en banc), rev'd on other grounds, 520 U.S. 17 (1997). In no case,

however, may the doctrine of equivalents ignore the individual claim elements. See Warner-Jenkinson, 520 U.S. at 40 (requiring "a special vigilance against allowing the concept of equivalence to eliminate completely any such [individual] elements").

Because "crystalline" in claims 1-5 is limited to "Crystal A" as defined by the seven PXRD peaks enumerated in claim 1 and in the specification of the '507 patent, the doctrine of equivalents cannot capture crystals that are not themselves equivalent to Crystal A. In turn, the bounds of Crystal A equivalents cannot ignore the limits on Crystal A in the '507 patent, which as discussed above, includes a conscious decision to distinguish Crystal B from the claimed invention. To recall, the applicant removed Crystal B from the US prosecution of the parent JP '199 application. The '507 patent indisputably describes and claims Crystal A, and not Crystal B. The '507 patent, of course, could have claimed the known Crystal B formulation which was known to the inventors because it appeared in their priority JP '199 application. The applicants chose not to claim Crystal B. Thus Crystal B compounds, most relevantly cefdinir monohydrate, fall outside the scope, literal or equivalent, of claims 1-5 of the '507 patent.

The parties agree that the "bulk" of Lupin's cefdinir product is Crystal B, not Crystal A. The degree to which Lupin's product may or may not also contain Crystal A is the central inquiry regarding the alleged literal infringement of claim 1, which is not part of the present appeal. Abbott cannot extend its exclusive right in the '507 claims under the doctrine of equivalents to embrace known but unclaimed subject matter. In other words, Abbott effectively disclaimed Crystal B during prosecution of the '507 patent, by removing the Crystal B disclosure from the parent JP '199 application and

emphasizing the sole teaching of Crystal A in communications with the PTO as well as in the '507 specification itself. Abbott cannot now recapture that unclaimed subject matter under the doctrine of equivalents because the Eastern District properly interpreted claims 2-5 to limit "crystalline" to Crystal A. To expand that claim term to embrace Crystal B would ignore the specific claim limitations of the '507 patent.

Alternatively this court notes that this case seems to fit within the dedication doctrine that forecloses invocation of the doctrine of equivalents. The patent applicant clearly knew of the Crystal B forms of the claimed invention because it claimed and disclosed them in its Japanese priority application. Yet it declined to claim an embodiment expressly disclosed in its priority document, thus dedicating that embodiment to the public and foreclosing any recapture under the doctrine of equivalents. See Johnson & Johnston Assocs. v. R.E. Serv. Co., 285 F.3d 1046, 1054 (Fed. Cir. 2002).

During prosecution, Abbott chose to eschew Crystal B and focus exclusively on Crystal A compounds. Without a complete record and no arguments about validity before this court on appeal, this court cannot speculate on the reasons for this choice. Nonetheless, the parties hotly contest whether Example 14, which reports obtaining "crystals" not specifically identified or described, and/or Example 16 of the '334 patent enable cefdinir monohydrate, i.e. Crystal B type crystals.

Beyond the attempt to reinflate the claims to encompass Crystal B based on mathematical comparisons of the PXRD peak patterns of Crystal A and Crystal B, Abbott also asserts that Lupin effectively admitted infringement by equivalents when it claimed before the Food and Drug Administration that its cefdinir generic was a

bioequivalent to Abbott's Omnicef product. While bioequivalency may be relevant to the function prong of the function-way-result test, bioequivalency and equivalent infringement are different inquiries. Bioequivalency is a regulatory and medical concern aimed at establishing that two compounds are effectively the same for pharmaceutical purposes. In contrast, equivalency for purposes of patent infringement requires an element-by-element comparison of the patent claim and the accused product, requiring not only equivalent function but also equivalent way and result. Different attributes of a given product may thus be relevant to bioequivalency but not equivalent infringement, and vice versa. As the Northern District of Illinois observed in the Sandoz case, "[i]f bioequivalency meant per se infringement, no alternative to a patented medicine could ever be offered to the public during the life of a patent." Sandoz PI Order, 486 F. Supp. 2d at 776. Thus, while potentially relevant, the bioequivalency of an accused product with a product produced from the patent at issue is not sufficient to establish infringement by equivalents.

Because Crystal B is not an equivalent of Crystal A, the Eastern District of Virginia did not err in granting summary judgment of noninfringement of claims 2-5, both with respect to literal and equivalent infringement, and with respect to equivalent infringement of claim 1.

IV.

This court reviews the grant or denial of a preliminary injunction for abuse of discretion. Amazon.com, Inc. v. Barnesandnoble.com, Inc., 239 F.3d 1343, 1350 (Fed. Cir. 2001). A district court may enter a preliminary injunction based on its consideration of four factors: "(1) the likelihood of the patentee's success on the merits; (2) irreparable

harm if the injunction is not granted; (3) the balance of hardships between the parties; and (4) the public interest." Erico Int'l Corp. v. Vutec Corp., 516 F.3d 1350, 1353-54 (Fed. Cir. 2008) (quoting PHG Techs., LLC v. St. John Cos., Inc., 469 F.3d 1361, 1365 (Fed. Cir. 2006).

Sandoz and Teva's Omnicef generic products, like Lupin's, are also at least primarily cefdinir monohydrate, a Crystal B compound. Sandoz PI Order, 486 F. Supp. 2d at 769. Before the Northern District of Illinois, the parties to the Sandoz litigation disputed whether Sandoz and Teva's products also contained small amounts of cefdinir anhydrate, i.e., Crystal A, which would fall within the literal scope of claim 1 of the '507 patent. Working primarily from the Eastern District of Virginia's claim construction, to which the parties to the Sandoz litigation agreed would bind their litigation as well for purposes of the preliminary injunction motion, the Northern District of Illinois denied Abbott's motion for a preliminary injunction, finding that Abbott was unlikely to prevail on the merits at trial.

This court detects no abuse of discretion in the Northern District of Illinois' preliminary injunction denial. As described above, the '507 patent is properly construed to exclude Crystal B, both as to literal and equivalent infringement. Thus, this court need not delve into the Northern District of Illinois' clarifications of the Eastern District of Virginia's claim constructions. The Northern District of Illinois succinctly concluded: "[w]e know that Crystal B was known to the plaintiffs because it had been included in the Japanese '199 patent. Thus we conclude that the plaintiffs deliberately excluded from the definition of Crystal A, cefdinir monohydrate, which is Crystal B." Id. at 775.

As to the alleged presence of small amounts of Crystal A in Sandoz and Teva's products, Abbott's evidence did not persuade the Northern District of Illinois. Id. This court perceives that decision as well within the trial court's discretion. As additional support, the Northern District observed that there was no evidence that any trace amounts of cefdinir anhydrate, i.e. Crystal A, in Sandoz and Teva's products "could be a contributing factor in the efficacy" and that even "if there is a small amount of cefdinir anhydrate in defendants' products, we do not conclude that this could cause literal infringement." Id. While these may be misstatements of the law, because de minimis infringement can still be infringement, see 35 U.S.C. § 271(a); see also SunTiger, Inc. v. Sci. Res. Funding Group, 189 F.3d 1327, 1336 (Fed. Cir. 1999) ("If a claim reads merely on a part of an accused device, that is enough for infringement."), this court need not reach that issue in a preliminary injunction context which affords the trial court broad leeway to discern a "likelihood of success." Likewise the district court may have overstated the relevance of efficacy, because the '507 patent contains no claim limitations relating to efficacy. But these misstatements were harmless because they merely formed an alternative basis for the Northern District of Illinois' reasonable assessment of the evidence proffered by Abbott for its preliminary injunction motion. As noted, this court sustains the trial court's discretion based primarily on its administration of the proper claim construction and its finding that Abbott was not likely to show Sandoz and Teva's products contained any Crystal A at all.

CONCLUSION

The Eastern District of Virginia correctly construed the '507 patent's recitation of "crystalline" in each of the asserted claims as limited to Crystal A, as outlined in the

specification. Because Abbott scrubbed all references to Crystal B in the '507 patent's specification, which were present in the '507 patent's parent foreign application, Abbott clearly demonstrated its intent to limit the '507 patent to Crystal A. This intent was further underscored by comments made during prosecution. As such, Abbott is unable to recapture Crystal B through broad claim language or under the doctrine of equivalents. The Eastern District of Virginia therefore properly concluded on summary judgment that Lupin's cefdinir product did not infringe claims 1-5 literally or claims 2-5 by equivalency. Similarly, the Northern District of Illinois did not abuse its discretion in declining to enter a preliminary injunction against Sandoz and Teva's cefdinir products.

AFFIRMED

COSTS

Each party shall bear its own costs.

United States Court of Appeals for the Federal Circuit

2007-1400

ABBOTT LABORATORIES,

Plaintiff-Appellant,

and

ASTELLAS PHARMA, INC.,

Plaintiff-Appellant,

v.

SANDOZ, INC.,

Defendant-Appellee,

and

SANDOZ GMBH,

Defendant,

and

TEVA PHARMACEUTICALS USA, INC. and
TEVA PHARMACEUTICAL INDUSTRIES, LTD.,

Defendants-Appellees,

and

RANBAXY LABORATORIES, LTD. and RANBAXY, INC.,

Defendants,

and

PAR PHARMACEUTICAL COMPANIES, INC. and PAR PHARMACEUTICAL,

Defendants.

2007-1446

LUPIN LIMITED,

Plaintiff/Counterclaim Defendant-Appellee,

and

LUPIN PHARMACEUTICALS, INC.,

Counterclaim Defendant-Appellee,

v.

ABBOTT LABORATORIES,

Defendant/Counterclaimant-Appellant,

and

ASTELLAS PHARMA, INC.,

Defendant/Counterclaimant-Appellant.

Appeals from the United States District Court for the Northern District of Illinois in case no. 07-CV-1721, Judge Wayne R. Andersen and the United States District Court for the Eastern District of Virginia in case no 3:06-CV-400, Judge Robert E. Payne.

NEWMAN, Circuit Judge, with whom Circuit Judges MAYER and LOURIE join, dissenting from en banc Section III.A.2.

The court today acts en banc to overturn a century of precedent and practice, and holds that a new product that is difficult to describe without reference to how it was made, but that is nonetheless a new and unobvious product, cannot be protected as a product if its description is aided by reference to how it was made. Heretofore a new product whose structure was not fully known or not readily described could be patented

as a product by including in the product description sufficient reference to how it can be made, to distinguish the new product from prior art products. Patentability was determined as a product, independent of any process reference in the claim, and validity and infringement were based on the product itself. This expedient for patenting products whose structure was not fully known at the time of filing the patent application has been called the "rule of necessity." It was pragmatic, fair, and just, for it attuned patent law and practice to the realities of invention.

Today the court rejects this expedient and discards this practice, ruling that all claims containing a process term under the rule of necessity now must be construed, for purposes of infringement, as limited to use of any process term that was used to assist in defining the product. That is, such a product is not patented as a product, however it is produced, but is limited to the process by which it was obtained. This is a new restraint on patents for new products, particularly today's complex chemical and biological products whose structure may be difficult to analyze with precision. It is a change of law with unknown consequences for patent-based innovation.

The court acts sua sponte, without explanation of what policy is intended to be served by this change, without consideration of the technologies that may be adversely affected by elimination of this expedient, without notice to those whose property rights may be diminished. In so doing, the court departs from statute, precedent, and practice. This change is as unnecessary as it is flawed, gratuitously affecting inventions past, present, and future. I respectfully dissent.

DISCUSSION

For most product inventions, the process by which the product was made, whether or not the process is itself a patentable invention, is not stated in the product claims. However, as the variety and complexity of invention and technology have increased, various forms of product claims with process terms have been used in specific circumstances, depending on the nature of the invention.[1] The form here at issue relates to product claims for new and unobvious products whose structure is not fully known, and for which process parameters are used to aid in defining the product. This claiming expedient has been recognized since at least 1891.

The court today overturns this expedient for all circumstances, brooking no exception. Acting en banc for the purpose, the court rules that if any process term or descriptive aspect is included in a product claim to aid in distinguishing a new product, the claim cannot be infringed by the identical product unless the same process aspect is used in making the accused product. The court holds that it is irrelevant whether the product is new or was known, irrelevant whether the product could have been fully described by its structure at the time of the patent application, irrelevant whether the particular invention is a new product or is actually a process. The court adopts a simplistic universal rule, thereby targeting a small but significant class of inventions. The effect of this decision on innovation in complex fields of science and technology is unknown to the court, for we have had no advice on the consequences of this change of

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[1] As discussed by Eric P. Mirabel, Product-By-Process Claims: A Practical Perspective, 68 J. Pat. & Trademark Off. Soc'y 3, 3-4 (1986), the various forms of product-by-process claims include "true" product-by-process claims, product claims with a process limitation, product claims with a process-derived structural element, and product claims with functional terms.

law. My dissent is directed as much to the court's procedure, as to the substance of the court's decision.

I

PROCEDURE

The court has given no notice of this impending en banc action, contrary to the Federal Rules of Appellate Procedure and contrary to the Federal Circuit's own operating procedures. The en banc court has received no briefing and held no argument, although the Federal Rules so require. The communities of inventors, innovators, and the public who may be affected by this change of law have had no opportunity to be heard. The court has received no information concerning the effect on patents that were granted based on this long-established practice, no advice on what kinds of inventions may now lie fallow because they are unprotected. Nor does the court explain its suspension of the standards of judicial process.

The Federal Rules have the force of law. 28 U.S.C. §2072. Federal Rules of Appellate Procedure 34 and 35 are here implicated. Rule 34 provides that "oral argument must be allowed in every case" unless certain specific exceptions exist:

> **Rule 34(a)(2) Standards.** Oral argument must be allowed in every case unless a panel of three judges who have examined the briefs and record unanimously agrees that oral argument is unnecessary for any of the following reasons:
>
> (A) the appeal is frivolous;
>
> (B) the dispositive issue or issues have been authoritatively decided; or
>
> (C) the facts and legal arguments are adequately presented in the briefs and record, and the decisional process would not be significantly aided by oral argument.

Applying the Rule 34 standards, it is clear that (A) this appeal is not frivolous and (B) the dispositive issue has not been authoritatively decided, for it is currently being addressed

en banc. There has been (C) no briefing and no record to the court, and this is not a case in which the decisional process would not be aided by oral argument. The en banc court has heard no argument, and has received neither written nor oral exploration of the diverse aspects of this long-established claiming practice.

Federal Rule of Appellate Procedure 35 has also failed of compliance. That rule recognizes the exceptional nature of en banc hearing or rehearing, and identifies the two circumstances warranting such procedure:

> **Rule 35(a)** . . . An en banc hearing or rehearing is not favored and ordinarily will not be ordered unless:
>
> (1) en banc consideration is necessary to secure or maintain uniformity of the court's decisions; or
>
> (2) the proceeding involves a question of exceptional importance.

When an en banc hearing or rehearing is ordered sua sponte by the court, whether for uniformity of decision or on a question of exceptional importance, the hearing or rehearing must receive the appellate process set by the Rules.

I agree that en banc review is appropriate, for this apparent conflict in our precedent has existed since 1992. Now that the court has undertaken to resolve the conflict, the withholding of public notice, or even notice to the parties to this case, is devoid of justification. The question is of importance, but there has been no assertion of urgency sufficient to require bypassing the standard appellate procedures. The breadth of the en banc court's ruling, the solidity of the precedent now overruled, the importance of the technologies affected, and the untold issued patents that are now placed in limbo, require this court's compliance with Federal Rules 34 and 35.

The Federal Circuit has recognized that it can benefit from the advice of those knowledgeable in the law and its purposes, in the areas of our nation-wide

responsibility. Patent law has a direct impact on innovation, industry, and technological advance, and when an en banc ruling may change the law affecting some areas of technology and the industries based thereon, this court has routinely sought to be informed, by the parties and amici curiae, of relevant concerns. When the impact of a sua sponte change of law transcends the interests of the parties to the specific case, notice to the interested public, as well as to the parties, is fundamental to due and fair process. The Federal Circuit's Internal Operating Procedure (IOP) 14 was adopted to implement these principles:

> IOP 14.3(c) If the sua sponte petition for hearing en banc is granted, a committee of judges appointed by the chief judge, which shall normally include the judge who initiated the poll, shall within seven working days (fourteen working days between June 21 and September 11) transmit on a vote sheet to the judges who will sit en banc an order setting forth the questions proposed to be addressed by the court en banc. The clerk shall provide notice that a majority of the judges in regular service has acted under 28 U.S.C. §46 and Fed. R. App. P. 35(a) to order the appeal to be heard en banc, and indicate any questions the court may wish the parties and amici to address. Notice shall be given that the court en banc shall consist of all circuit judges in regular service who are not recused or disqualified. Additional briefing and oral argument will be ordered as appropriate.

United States Court of Appeals for the Federal Circuit, Internal Operating Procedures at 40, available at http://www.cafc.uscourts.gov/pdf/IOPs122006.pdf. This IOP has not been followed. No notice was given, even to the parties, that the court had ordered this question to be reheard en banc; nor did the court advise the parties or the public as to the aspects being addressed for en banc decision. The court is acting sua sponte, without notice and without argument and without an opportunity for participation. By bypassing this court's standard operating procedure, as well as violating the Federal Rules of Appellate Procedure, the court has deprived itself of input concerning the

experience of precedent, of advice as to how this change of law may affect future innovation, and of guidance as to the effect on existing property rights.

II

PRECEDENT AND PRACTICE

The court's opinion does not mention the long-established precedent that it is overturning. This is not a simple conflict between isolated rulings of the Federal Circuit; it is a change of law and practice with roots in century-old decisions. I start with this precedent, for the expedient of what came to be called the "rule of necessity" originated in the recognition, by the courts and the Patent Office, that not all new products could be fully described by their structure, due to the state of scientific knowledge or available analytical techniques. It was also recognized, over a century ago, that sufficient distinction from prior art products could sometimes be achieved by reference to how the product was made. Thus the courts and patent administrators established the exception that permitted inclusion in a product claim of sufficient recitation of how the product was made, to aid in identifying the product and distinguishing it from the prior art. This claim form was loosely called a "product-by-process" form, although that term includes a variety of situations, see n.1 supra, having diverse legal consequences. The only form here at issue is that in which the product is new and its structure is not fully or readily known, such that its definition as a product is aided by referring to how it was made. Since before 1891, this has been an accepted way to claim products as products, recognizing that this is an exception to the general rule that new products are claimed without reference to the process by which they are produced.

This exception was discussed in 1891 in Ex parte Painter, the Commissioner of Patents explaining that when there is entitlement to a patent on a new article of manufacture, it can be claimed by reference to the process of producing it, when the inventor lacks other language to "define and discriminate" the invention:

> It requires no argument to establish the proposition that as a rule a claim for an article of manufacture should not be defined by the process of producing that article. On the other hand, when a man has made an invention his right to a patent for it, or his right to a claim properly defining it, is not to be determined by the limitations of the English language. When the case arises that an article of manufacture is a new thing, a useful thing, and embodies invention, and that article cannot be properly defined and discriminated from prior art otherwise than by reference to the process of producing it, a case is presented which constitutes an exception to the rule.

1891 C.D. 200, 200-01 (Comm'r Pat. 1891). The Commissioner cited, as an earlier example of this exception, the claim in Globe Nail Co. v. U.S. Horse Nail Co., 19 F. 819 (C.C.D. Mass. 1884) (sustaining validity of claim directed to horse-shoe nail claimed by reference to its process of manufacture, and finding it infringed by the accused nail having only a "trivial and unsubstantial variation" from the claimed product). In contrast, where the patent application made clear that the product could be described by its structure, the Patent Office ruled that the exception did not apply. See, e.g., Ex parte Scheckner, 1903 C.D. 315, 315-16 (Comm'r Pat. 1903) (sustaining rejection of claim directed to an etched printing-plate that "specifies certain steps by means of which the etching is accomplished" because other claims "define the plate in terms of its structure").

This expedient has been discussed in various judicial decisions. In all cases the issue has not been whether this expedient was available, for its availability was not challenged; the issue was simply its application to the particular facts. For example, at

a time when it heard direct appeals from Patent Office rulings, the D.C. Circuit remarked on this "only exception" to the general rule of product claiming, stating:

> It is a well-settled rule of patent law that claims for a product which is defined by the process of producing it will not be allowed; and the only exception to this rule seems to be in cases where the product involves invention and cannot be defined except by the process used in its creation. In extreme cases of this character, the product may be allowed; but that is not this case, especially in view of the broad claims allowed appellant in his copending application . . . .

In re Brown, 29 F.2d 873, 874 (D.C. Cir. 1928) (emphasis added).

The Court of Customs and Patent Appeals discussed precedent involving claims for processes and products in various factual situations, and summarized that:

> Where it is possible to define a product by its characteristics, the practice is clearly settled that this should be done. Where, however, the product is novel and involves invention and cannot be defined except by the steps of the process involved in its creation, there are cases holding that such a claim may be allowed, and it has been sustained by a Court.

In re Butler, 37 F.2d 623, 626 (CCPA 1930) (emphasis added) (quoting Ex Parte Feisenmeier, 1922 C.D. 18 (Comm'r Pat. 1922)). The CCPA then found this rule inapplicable to the facts of Butler's invention, explaining that "the record at bar does not meet this requirement [that the product was new]." Id.

In In re Lifton, 189 F.2d 261 (CCPA 1951), the CCPA again commented on this exception for product claims, stating that when "proper article claims" were possible they must be used, with the exception of when such claims are "impossible":

> This court has uniformly held that a claim for an article must define the article by its structure and not by the process of making it. The one exception to this rule, where the invention is the article and it is impossible to otherwise define it, is clearly ruled out in the present case because appellant has demonstrated the possibility of proper article claims by including several devoid of process limitations.

Id. at 263 (emphasis added, citations omitted). The court again recognized "the one exception," holding once again that it does not apply when the product can be described independently of the process of making it.

These inquiries into the facts warranting application of the exception demonstrate that the rule of necessity was seldom applied, but was nonetheless recognized both by the courts and the Patent Office. Decisions of the Patent Office Board of Appeals illustrate the practice. See, e.g., Ex parte Pfenning, 65 U.S.P.Q. 577 (Pat. Off. Bd. App. 1945) (allowing claim "directed to a product which results from the method of claim 9" in light of applicant's argument that "it is impossible in the instant case to define the product adequately in terms of the elements which compose it or in terms of its physical characteristics"); Ex parte Lessig, 57 U.S.P.Q. 129 (Pat. Off. Bd. App. 1943) (allowing claim for a "product containing vulcanized rubber" strongly adhered to fibers which "has been prepared by the process of claim 4" because "it is not possible to otherwise distinguish over the art of record").

Commentators have explained that this claiming practice became of increasing importance as the complex sciences blossomed. See, e.g., Mark D. Passler, Product-by-Process Patent Claims: Majority of the Court of Appeals for the Federal Circuit Forgets Purpose of the Patent Act, 49 U. Miami L. Rev. 233, 233 n.3 (1994) ("Such claims are often used by companies to patent complex drug or chemical products whose structure is not completely understood and, therefore, can only be accurately described by the process through which it is made."). It is well known that the full structure of some chemical and biological products is not always known at the time the

patent application is filed. Indeed, it is a tenet of the scientific method that explanation and theory tend to follow, not precede, the observation of a development in the science.

The CCPA continued to recognize the use of process terms to aid in describing new products—the form of claim sometimes called a "pure" product-by-process claim, see n.1 supra—and repeatedly ruled that such claims are properly viewed as product, not process, claims. The court also disallowed such claims where the product itself was not novel and unobvious. The court confirmed that such a claim, when justified for a novel and unobvious product, is properly construed as encompassing the full scope of a product claim. For example, in In re Bridgeford, 357 F.2d 679 (CCPA 1966), the court again explained that a new product may be defined by the process of making it if there is no other way to describe the product, stressing that "the invention so defined is a product and not a process," id. at 682. The Bridgeford court relied on this view of the scope of the product-by-process claims in a related patent, and held unpatentable for double patenting claims that defined the "product per se." Id. at 680. The court explained that product-by-process claims are true product claims, and overruled the suggestion in In re Freeman, 166 F.2d 178, 181 (CCPA 1948), that product-by-process claims are "dependen[t] . . . on process limitations" and therefore coextensive with process claims. Bridgeford, 357 F.2d at 683 n.6 ("While there is some language in Freeman to support the contention that a product-by-process type claim differs only 'in scope' from a process type claim and they therefore 'are directed to a single invention,' (166 F.2d at 181) so far as this is inconsistent with our holding here it must be overruled.").

My colleagues misstate the holding of Bridgeford, for Bridgeford directly contravenes today's holding. In Bridgeford the CCPA noted that "some courts" have construed claims with process steps as limited to the recited process, id. at 683 n.5, apparently without inquiring whether the rule of necessity justified full product scope for the invention at issue. The CCPA's observation that patents are construed inconsistently in other courts cannot be taken, as apparently do my colleagues, as error by the CCPA. To the contrary, the inconsistency among courts led eventually to consolidation.[2]

Again in In re Brown, 459 F.2d 531 (CCPA 1972), the CCPA explained that product-by-process claims are product claims, not process claims, and that the patentability of the product must be established independently of the process by which it is identified. See id. at 535 ("[I]n spite of the fact that the claim may recite only process limitations, it is the patentability of the product claimed and not of the recited process steps which must be established.").

Other decisions discussing application of this expedient to claims directed to complex new products include In re Pilkington, 411 F.2d 1345, 1349 (CCPA 1969) ("While we are satisfied that the references of record do not anticipate appellant's glass or demonstrate that it would be obvious, the differences between that glass and the glass of the prior art do not appear to us to be particularly susceptible to definition by the conventional recitation of properties or structure."), and In re Fessmann, 489 F.2d

[2] The Hruska Commission Report, which informed the debate that eventually led to the formation of our court, described the varying attitudes towards patents held by the regional courts of appeal and the variations in patent rulings among the circuits. See Commission on Revision of the Federal Court Appellate System Structure and Internal Procedures: Recommendations for Change, 67 F.R.D. 195, 370 (1975).

742, 743 (CCPA 1974) (affirming obviousness rejection of product-by-process claim directed to a "liquid smoke" product, but observing that prior art compositions "are complex mixtures of the chemical compounds which can be derived from wood" which "defy simple characterization and this fact presumably accounts for the use of product-by-process claims"). The need for this expedient, and the proper scope afforded such claims, is summarized in the treatise Walker on Patents:

> [P]atent rights over a chemical product are typically independent of the process by which the product is made, and are particularly valuable because of this fact. This independence is normally accomplished by defining the product in terms of its structural features alone, with no reference in the claims to process steps whatsoever. The state of chemical technology, however, is sometimes too limited for a structural description of this type to be made. The structure of some chemicals, especially those including elaborate polymer chains, cannot be accurately determined. The same chemicals, however, may be both economically valuable and technologically reproducible, in the sense that they can be reliably made by subjecting a particular set of raw materials to a particular set of process steps.
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> * * *
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> The law reacted to these difficulties by making it easier to obtain traditional product protection over this special class of chemicals. The inventor was allowed to describe such a chemical in terms of how one gained possession of it, that is, by way of the process steps by which it was made. Once he did so, the law preserved to the inventor the fullest measure of product-only protection that it could; it treated the process recitations as proxies for the direct recitations of structure that could not be made. Such a claim was therefore equivalent to one stated in terms of structure only. It would broadly dominate all methods by which the chemical could be made or used. At the same time, it carried the same dangers of running afoul of the art: it would be anticipated if the chemical had been produced previously, even if by a method other than what the inventor disclosed.

1 Moy's Walker on Patents §4:74 (4th ed. 2008) (emphases added).

The en banc court appears to misunderstand this precedent, for my colleagues now state that "binding case law" of the Court of Customs and Patent Appeals and the Court of Claims mandates a single rule for all claims that contain any process terms,

whether the product is novel or known, citing In re Hughes, 496 F.2d 1216 (CCPA 1974), for this proposition. However, Hughes does not state this proposition; Hughes stands for the contrary proposition. In Hughes the question was the patentability of claims directed to "shakes" as are used in roofing, as follows:

> 12. Shakes manufactured from a shake bolt by the process of making a plurality of cuts into and across the shake bolt to an extent to establish predetermined tip lengths, and splitting the weather end portions of the shakes from the bolt by starting the splits at the inner ends of the cuts and continuing the splits to the end of the bolt.

This claim had been rejected as an improper product-by-process claim, on the ground that the product could be described without including process steps. The Hughes court acknowledged the general rule against product-by-process claiming, but also explained the "proper exception to the general rule" as first set forth in Painter, as follows:

> [T]he Commissioner of Patents enunciated the general rule that a product should not be defined in terms of the process of making it. In Painter, a proper exception to the general rule was found on the ground that the product could not be properly defined and discriminated from the prior art otherwise than by reference to the process of producing it. This basic rule and the exception have been recognized and followed continuously by the Patent Office and the Courts.

Hughes, 496 F.2d at 1218 (quoting approvingly the Solicitor's argument). The court reaffirmed that "in spite of the fact that a product-by-process claim may recite only process limitations, 'it is the product which is covered by the claim and not the recited process steps.'" Id. Contrary to my colleagues' statement, Hughes did not eliminate this form of claim, or change its role as a product claim. Indeed, the Hughes court applied the exception and reversed the Board's rejection of a product-by-process claim, stating:

> We agree with appellant that the [general] rule should not be applied to the situation before us. We have been shown no true product claim which describes appellant's invention, in the words of the solicitor, "in terms of structure or physical characteristics." When an applicant seeks to

> describe his invention by a product-by-process claim because he finds that his invention is incapable of description solely by structure or physical characteristics, it is incumbent upon the Patent Office to indicate where, or how, the applicant's invention is, or may be, so described.

Id. at 1219. My colleagues could hardly have selected less apt support for their construction of product-by-process claims, for Hughes explicitly states that such claims are for the product, not the process.

In addition to misstating precedent of the CCPA, the en banc court also mischaracterizes the decisions of our predecessor the Court of Claims, stating that the Court of Claims' decisions support today's ruling. The court cites Tri-Wall Containers v. United States, 408 F.2d 748 (Ct. Cl. 1969), for this purpose. That citation, too, is mysterious, for in Tri-Wall Containers the court found that the claimed product was not "new" because it had been on sale for more than the permitted period, although the product that was on sale had been made by a different process than the process stated in the claim. The Court of Claims stated that the evidence showed that "the prior art product and the claimed product are structurally identical," id. at 751, and explained that a known product cannot be patented by including process terms in the claim:

> It is well established that a product claimed as made by a new process is not patentable unless the product itself is new. The Wood-Paper Patent, 90 U.S. (23 Wall.) 566, 596, 23 L. Ed. 31 (1874), Cochrane v. Badische Anilin & Soda Fabrik ["BASF"], 111 U.S. 293, 311, 4 S. Ct. 455, 28 L. Ed. 433 (1984). . . .
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> . . . .
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> More recent cases point out that the addition of a method step in a product claim, which product is not patentably distinguishable from the prior art, cannot impart patentability to the old product. Jungerson v. Baden, 69 F. Supp. 922, 928 (D.C.S.D.N.Y. 1947), aff'd, 166 F.2d 807 (2d Cir. 1948), aff'd, 335 U.S. 560, 69 S. Ct. 269, 93 L. Ed. 235 (1949); In re Stephens, 345 F.2d 1020, 1023, 52 CCPA 1409 (1965).

Tri-Wall Containers, 408 F.2d at 750-51. This case applied the standard rule that old products cannot be patented—it contains no statement limiting the scope of claims that include process aspects to aid in describing new products. The Supreme Court cases cited in Tri-Wall are all directed to new processes for making old products—these are the same cases that the en banc court today incorrectly applies to new products, as I discuss post.

Contrary to my colleagues' statement, CCPA and Court of Claims precedent do not support today's en banc thesis. Our predecessor courts understood the complexity of patenting, and the CCPA consistently implemented the expedient whereby process terms contributed to the description of complex new products of incompletely known structure. These courts recognized the independence of product claims for new products, and did not limit such claims to the specific process steps that were used to aid in describing the product.[3]

With the advent of the Federal Circuit, this court continued to apply these principles. In In re Thorpe, 777 F.2d 695 (Fed. Cir. 1985), the court explained that

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[3] The en banc court impugns the CCPA's experience. Maj. op. at 18 (stating that the CCPA had "virtually no jurisdiction to address infringement litigation"). The CCPA for many years addressed infringement litigation, in appeals from the International Trade Commission and its predecessor tribunals. E.g., Sealed Air Corp. v. Int'l Trade Comm'n, 645 F.2d 976 (CCPA 1981) (issues of validity and infringement); Hale Fire Pump Co. v. Tokai, Ltd., 614 F.2d 1278 (CCPA 1980) (issues of validity, scope, and infringement); In re Orion, 71 F.2d 458 (CCPA 1934) (issues of jurisdiction and infringement).

Our predecessor's legal and scholarly distinction in the field of patent law, and the high regard in which Congress and the innovation communities held the jurisprudence of the CCPA were a critical foundation for formation of the Federal Circuit and its charge to reinvigorate the role of the patent system in service to the nation's technological innovation. See 125 Cong. Rec. 23,462 (1979) (statement of Sen. DeConcini) ("It is a reflection of high esteem which Congress has for the sitting judges of the Court of Claims and Court of Customs and Patent Appeals that these judges will become the first judges of the new Court of the Federal Circuit.").

product-by-process claims are anticipated when the product existed in the prior art, even if the product was made by a different process. My colleagues are mistaken in stating that Thorpe held that all such claims are to be construed as process claims, even when the product is new and the rule of necessity justifies this mode of describing the invention. In Thorpe the product was not new; it was a known color developer for carbonless paper copy systems, and this court held that the PTO correctly rejected the claim to "the product of the process of claim 1," explaining that since the product was old it could not be claimed as a product, whether or not process steps are recited in the claim.

The facts of Thorpe did not concern the exception and expedient where process terms are invoked to describe a new product of complex structure. This exception is rarely invoked. The general rule requiring claims to have a process-free definition of the structure of a new product accommodates most inventions. Some recent exceptions are seen in emerging aspects of biotechnology. For example, in Amgen, Inc. v. Chugai Pharmaceuticals Co., 706 F. Supp. 94 (D. Mass. 1989), aff'd in relevant part, 927 F.2d 1200 (Fed. Cir. 1991), the district court considered the following claim:

> 4. A procaryotic or eucaryotic host cell transformed or transfected with a DNA sequence according to claim 1, 2 or 3 in a manner allowing the host cell to express erythropoietin.

Id. at 108. The district court found claim 4 "ambiguous," explaining that while it is directed to a new product—this host cell—the words "transformed or transfected" appear to invoke a process. The district court recognized that "[i]n the traditional patent framework, a product is wholly separate and distinct from a process." Id. at 107. The court observed that "[a] product patent gives the patentee the right to restrict the use

and sale of the product regardless of how and by whom it was manufactured," while "[a] process patentee's power extends only to those products made by the patented process." Id. (quoting United States v. Studiengesellschaft Kohle, 670 F.2d 1122, 1127-28 (D.C. Cir. 1981)). The district court, affirmed by the Federal Circuit, found this claim to be valid and infringed as a product claim, and although many issues and arguments were present in this litigation, the applicability of the venerable rule of necessity was not at issue.

In Scripps Clinic & Research Foundation v. Genentech, Inc., 927 F.2d 1565 (Fed. Cir. 1991), the Federal Circuit addressed the interpretation and scope of claims exemplified by claim 13:

> 13. Highly purified and concentrated VIII:C prepared in accordance with the method of claim 1.

Claim 1 set forth the method referred to in claim 13, as follows:

> 1. An improved method of preparing Factor VIII procoagulant activity protein [VIII:C] comprising the steps of
> (a) adsorbing a VIII:C/VIII:RP complex from a plasma or commercial concentrate source onto particles bound to a monoclonal antibody specific to VIII:RP,
> (b) eluting the VIII:C,
> (c) adsorbing the VIII:C obtained in step (b) in another adsorption to concentrate and further purify same,
> (d) eluting the adsorbed VIII:C, and
> (e) recovering highly purified and concentrated VIII:C.

It was not disputed that the product was a new product, that the "highly purified and concentrated" blood clotting Factor VIII:C had not previously been obtained, and that a complete structural identification of Factor VIII:C was not available. The defendant Genentech had made its commercial Factor VIII:C not by the method set forth in claim 1, but by using a sample of the Scripps product to "clone" Factor VIII:C protein using

recombinant DNA techniques. One question presented in the case was whether claims such as claim 13 were infringed by the same product produced by a different method, or whether such claims were infringed only if the accused infringer used the process of claim 1.

Scripps stressed that its product was novel and enabled and was patentable as a product, although the full structure of Factor VIII:C was not available at that stage of the science. The court addressed whether claims exemplified by claim 13, properly construed, were product claims, or whether they were limited to the specific processes in the process claims to which they referred. This court held that the claims were product claims. The court held that since claims are construed the same way for infringement as for validity, the question was whether the Genentech product was the same as the claimed product, not whether they were produced by the same process. The court remanded to the district court for this factual determination. Scripps, 927 F.2d at 1584.

After Scripps was decided, a panel of this court decided an appeal concerning plastic innersoles for shoes. In Atlantic Thermoplastics Co. v. Faytex Corp., 970 F.2d 834 (Fed. Cir. 1992), the claims at issue were represented by:

Claim 24. The product produced by the method of claim 1.

In turn, claim 1 was as follows:

> 1. In a method of manufacturing a shock-absorbing, molded innersole for insertion in footwear, which method comprises:
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> (a) introducing an expandable, polyurethane into a mold; and
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> (b) recovering from the mold an innersole which comprises a contoured heel and arch section composed of a substantially open-celled polyurethane foam material, the improvement which comprises:
>
> (i) placing an elastomeric insert material into the mold, the insert material having greater shock-absorbing properties and being

> less resilient than the molded, open-celled polyurethane foam material, and the insert material having sufficient surface tack to remain in the placed position in the mold on the introduction of the expandable polyurethane material so as to permit the expandable polyurethane material to expand about the insert material without displacement of the insert material; and
>
> (ii) recovering a molded innersole with the insert material having a tacky surface forming a part of the exposed bottom surface of the recovered innersole.

The panel held that a claim in the form of claim 24 always requires use of the referenced method, and that it is irrelevant whether the product was new or known. The court stated that the rule of necessity, as applied in Scripps, is contrary to Supreme Court rulings. The panel stated that the decision in Scripps is incorrect. A majority of the Federal Circuit declined to resolve the conflict en banc, resulting in several further opinions. E.g., Atlantic Thermoplastics Co. v. Faytex Corp., 974 F.2d 1279 (Fed. Cir. 1992) (dissents of Chief Judge Nies and Judges Rich, Newman, and Lourie from denial of rehearing en banc). Judge Rich wrote:

> [T]his whole excursion was unnecessary because the patentee admitted that claim 24, the product-by-process claim, was limited to the process. The claim read: "The molded innersole produced by the method of claim 1." There was, therefore, no occasion to review the law to determine how the claim should be construed. . . . We are not here to provide restatements of the law. Such restatements should not be made without an opportunity for all affected parties to be heard from. The affected parties here are not the vendors of inner soles but largely the entire chemical industry, particularly the pharmaceutical manufacturers.

Id. at 1280 (Rich, J., dissenting from denial of rehearing en banc).

Most trial courts continued to recognize the rule of necessity. For example, in Trustees of Columbia University v. Roche Diagnostics GmbH, 126 F. Supp. 2d 16 (D. Mass. 2000), the district court considered claims such as the following.

> 72. A eukaryotic cell into which foreign DNA I has been inserted in accordance with the process of claim 54.

The court referred to the Scripps/Atlantic conflict, concluded that the earlier panel decision controlled under the Federal Circuit's rule, see Newell Companies, Inc. v. Kenney Manufacturing Co., 864 F.2d 757, 765 (Fed. Cir. 1988) ("This court has adopted the rule that prior decisions of a panel of the court are binding precedent on subsequent panels unless and until overturned in banc."), and applied the Scripps ruling, holding that the new cell was not limited by the process by which it was made.

The PTO also continued to apply the rule of necessity. In instructing examiners that products should whenever possible be described without reference to how they were made, the PTO continued to point out the exception that patentability as a product is not foreclosed when independent description is not available. The Manual of Patent Examining Procedure (MPEP) instructs the examiner to consider the structure implied by any process steps in the claim:

> The structure implied by the process steps should be considered when assessing the patentability of product-by-process claims over the prior art, especially where the product can only be defined by the process steps by which the product is made, or where the manufacturing process steps would be expected to impart distinctive structural characteristics to the final product.

MPEP §2113 (8th ed., July 2008 rev.). This has been the practice since at least Ex parte Painter in 1891. I am surprised at the en banc court's casual misstatement about "the treatment of product-by-process claims throughout the years by the PTO," maj. op. at 17, for the statement is directly contrary to the treatment of such claims throughout the years by the PTO.

The en banc court's insistence that one universal rule should now be applied is contrary to the entire body of decisional law, including the Supreme Court cases cited by my colleagues. As I next discuss, in most of the cited cases the product was not a

new product and thus was not patentable as a product, whether or not any process term was included in the claim. The Court consistently held that when the product was old and only the process was a patentable invention, a claim for the "product of that process" could not cover the old product made by a different process. That is, and has always been, the law. I comment briefly on the Court's cases that my colleagues misinterpret and misapply:

Cochrane v. BASF

The en banc opinion relies primarily on Cochrane v. Badische Anilin & Soda Fabrik, 111 U.S. 293 (1884) ("BASF"), even though my colleagues acknowledge that the product in that case was the well-known dye alizarine. The patent before the Court was a reissue patent that claimed artificial alizarine in the following way:

> Artificial alizarine, produced by either of the methods herein described, or by any other method which will produce a like result.

The Court held that since alizarine was a known product, the claim was limited to the patentee's two processes, stating:

> It was an old article. While a new process for producing it was patentable, the product itself could not be patented, even though it was a product made artificially for the first time, in contradistinction from being eliminated from the madder root. Calling it artificial alizarine did not make it a new composition of matter, and patentable as such, by reason of its having been prepared, artificially, for the first time, from anthracite, if it was set forth as alizarine, a well-known substance. Wood Paper Patent, 23 Wall. 560, 593 [(1874)]. There was therefore no foundation for reissue No. 4,321, for the product, because, on the description given, no patent for the product could have been taken out originally.

111 U.S. at 311-12. The Court accordingly limited the claim to the two processes described in the patent, and in the portion of BASF quoted by my colleagues, the Court discussed the proofs needed to show infringement:

> [U]nless it is shown that the process of [the specification] was followed to produce the defendants' article, or unless it is shown that the article could not be produced by any other process, the defendants' article cannot be identified as the product of the process of [the specification]. Nothing of the kind is shown.

Id. at 310. The Court did not state, or imply, despite my colleagues' contrary theory, that a claim to a new and complex product that is of necessity defined and distinguished by the process by which it was made, can never be infringed unless that specific process is practiced. There was no issue in BASF of a product that could not be defined without reference to how it was made. The BASF Court, providing guidance, remarked on the importance of independent description of a patented product, in the following sentence cited by my colleagues:

> Every patent for a product or composition of matter must identify it so that it can be recognized aside from the description of the process for making it, or else nothing can be held to infringe the patent which is not made by that process.

Id. at 310. This statement is indeed the general rule, as stated by the Patent Commissioner several years later in Ex parte Painter. However, BASF did not present the situation for which the expedient of necessity was created, for as the Court stated, the invention was "a process for preparing alizarine, not as a new substance prepared for the first time, but as the substance already known as alizarine, to be prepared, however, by the new process, which process is to be the subject of the patent, and is the process of preparing the known product alizarine from anthracine." Id. at 308-09.

This was not an instance of a new product describable only in terms of its process of manufacture. The BASF decision lends no support to today's en banc rule that every product claim that mentions a process step is always restricted to that

process, with no exception, no expedient, no preservation of the distinctions among forms of claim based on the nature of the invention.

The Goodyear Dental cases

The en banc court also states that its new ruling is supported by two cases relating to a patent on the use of vulcanized rubber to form a plate for holding dentures, Smith v. Goodyear Dental Vulcanite Co., 93 U.S. (3 Otto) 486 (1876), and Goodyear Dental Vulcanite Co. v. Davis, 102 U.S. (12 Otto) 222 (1880). Review of these cases reveals no support for the en banc court's statement of their holdings. The claim at issue was:

> The plate of hard rubber or vulcanite, or its equivalent, for holding artificial teeth, or teeth and gums, substantially as described.

Davis, 102 U.S. (12 Otto) at 223. The claim was written in the then-standard format of incorporating the description in the specification through the phrase "substantially as described." This was not a product-by-process or product-of-the-process claim at all, for the claim contains no process distinction or limitation, but simply refers to the description in the specification. Nonetheless, the en banc majority appears to state that these cases mean that the Supreme Court requires that all claims for products whose method of production is set forth in the specification—as is required by the description and enablement requirement—cannot be infringed unless that method is used.

That is not what the Goodyear Dental cases said. The Court referred to the position of Goodyear Dental Vulcanite that its patent covered all dental plates made of vulcanized rubber, and held, upon reviewing the specification and the prior art, that the process of manufacture was what distinguished this dental plate from the prior art dental plates, and concluded: "The invention, then, is a product or manufacture made in a

defined manner. It is not a product alone, separated from the process by which it is created." Smith, 93 U.S. (3 Otto) at 493. Were the claim not limited to this process, the Court concluded that the claim would not have been patentable. See id. at 492 (holding that if the patent were for a "mere substitution of vulcanite for other materials, which had previously been employed as a base for artificial sets of teeth" then it "constituted no invention"). Four years later, considering the same patent in Davis, the Court emphasized that the claim was limited to use of vulcanized rubber or its equivalent, and held that since the accused infringer made its dental plate with celluloid, there could not be infringement. See 102 U.S. (12 Otto) at 228-30.

The court today cites these cases as definitive of the interpretation of claims with process elements, although the only process referent is the phrase "substantially as described." This flawed reasoning was disposed of in 1890 in the classic Robinson on Patents, and until now has not reappeared:

> In stating Claims certain phrases are frequently employed to which a special importance seems to be attached by applicants. Among these are the phrase "substantially as described" and others of the same meaning. These phrases import the same thing when used in a Claim as when elsewhere employed. They are neither necessary nor technical. The reference they make to the Description is always implied, and relates only to the essential features of the invention as therein delineated. They add nothing, therefore, to the certainty of the Claim, nor do they detract from it unless the claimant carelessly inserts them as a substitute for a more clear and definite statement of his invention.

II W.C. Robinson, Robinson on Patents 517 (1890) (footnotes omitted).

Merrill v. Yeomans

My colleagues also rely on Merrill v. Yeomans, 94 U.S. (4 Otto) 569 (1877). Again, the relevance is remote. The Merrill Court explained that the issue was the "correct construction of plaintiff's patent," id. at 569, construing the following claim:

> [T]he above-described new manufacture of the deodorized heavy hydrocarbon oils, suitable for lubricating and other purposes, free from the characteristic odors of hydrocarbon oils, and having a slight smell like fatty oil, from hydrocarbon oils, by treating them substantially as hereinbefore described.

Id. at 570. The Court examined the specification to determine what was invented, and found that the invention was directed solely to a process, not to a product. The Court then concluded that the claim's usage "new manufacture" referred to the manufacturing process, and not to the product. The claim was thus a process claim, and no "product-by-process" issue was presented. The Court concluded that the defendant's oil, which was made by a different process, did not infringe.

The Merrill Court discussed its practice of looking to the patent application and interpreting the claim in light of what was "really invented":

> [W]here it appears that a valuable invention has really been made, this court, giving full effect to all that is found in the application on which the Patent Office acted, will uphold that which was really invented, and which comes within any fair interpretation of the patentee's assertion of claim.

Id. at 573. This approach is inimical to the en banc court's theory that it is irrelevant what the patentee describes as his invention, and that if a process step is mentioned in the claim or "substantially described" in the specification, the claim always requires performance of that step. Although the Court in Merrill was not confronted with a situation of indescribable product or necessity bred of complexity—indeed no product at all was claimed—neither did the Court hold that every product invention must be limited by the process that produced the product.

The Wood Paper Patent case

The list of Supreme Court cases relied on by my colleagues continues with The Wood Paper Patent, 90 U.S. 566, 596 (1874), where claims with the standard

"substantially as described" language were construed in two reissue patents relating to the pulping of wood to make paper. The Court explained that one reissue patent was for "a product or manufacture, and not for the process by which the product may be obtained," and the other "for a process and not for its product." Id. at 593. The Court examined the prior art and concluded that the claim for the product could not be sustained, because the product produced by the inventor's new pulping process was not new:

> Paper-pulp obtained from various vegetable substances was in common use before the original patent was granted to Watt & Burgess, and whatever may be said of their process for obtaining it, the product was in no sense new. The reissued patent, No. 1448, is, therefore, void for want of novelty in the manufacture patented.

Id. at 596. The Court then discussed the reissue patent for the "process and not for its product," and held this reissue void because it claimed a different invention than in the original patent. The Court also discussed several other patents directed to boilers used to produce paper-pulp, and to a process for bleaching straw. Nothing in this case concerns the product-by-process issue on which the court is today acting.

I cannot discern why the en banc court relies on The Wood Paper Patent case as invalidating Scripps, and the court has not attempted to explain.

Plummer v. Sargent

The en banc court also relies on Plummer v. Sargent, 120 U.S. 442 (1887), which again provides no support for my colleagues' thesis. This case again illustrates the Court's practice of reviewing what the patentee stated he invented as set forth in the specification in light of the prior art. The claim in Plummer was for a "new manufacture," "substantially as described":

> What I claim and desire to procure by letters patent is the new manufacture hereinabove described, consisting of iron ornamented in imitation of bronze by the application of oil and heat, substantially as described.

Id. at 445. The trial court had found non-infringement because the defendant had used a prior art process for bronzing iron. This prior process was work of F.W. Brocksieper, an employee of the defendant's predecessor company. The Supreme Court affirmed, stating that the claims were limited to the process described in the specification:

> It seems necessarily to follow from this view either that the Tucker patents are void by reason of anticipation practiced by Brocksieper, or that the patented process and product must be restricted to exactly what is described . . . .

Id. at 449. The Court thus limited the claims to the process described by the patentee, not because of any rule about limiting a product to how it was made in the specification, but to sustain validity of the patent in view of the Brocksieper prior art. The decision in Plummer is unrelated to any rule of claim construction based on whether process terms are included in the claim.

These nineteenth-century cases do not relate to the en banc court's new universal rule of claim construction, whereby all product claims having process terms are treated as process claims, whatever the nature of the product, whatever the need for process descriptors, or any other factor that precedent shows to be relevant to the exception that is here at issue as to the use of and construction of such claims. Nor do any more recent Court cases.

General Electric v. Wabash

My colleagues also cite General Electric Co. v. Wabash Appliance Corp., 304 U.S. 364 (1938), although the relevance of this case is, again, not apparent, for it

involved no product-by-process claims, but rather claims that recite the properties of the product. A typical claim is claim 25, which describes an electric lamp filament composed of tungsten grains of a size and shape that prevents sagging of the filament:

> 25. A filament for electric incandescent lamps or other devices, composed substantially of tungsten and made up mainly of a number of comparatively large grains of such size and contour as to prevent substantial sagging and offsetting during a normal or commercially useful life for such a lamp or other device.

Id. at 368. The Court held this claim "invalid on its face" for failing to provide a "distinct and definite statement of what he claims to be new, and to be his invention." Id. at 369. The Court stated that the description of the grains as "of such size and contour as to prevent substantial sagging and offsetting" was "inadequate as a description of the structural characteristics of the grains." Id. at 370. The Court also criticized the use of functional language in the claim, stating that such terms were too indefinite to provide clear guidance. Id. at 371. There was no issue of whether process steps in the claims were regarded as limiting, for there were no process steps in the claims. Instead, the Court stated that even the implicit inclusion of process steps could not save the claim, because the description of the process in the specification was inadequate:

> Even assuming that definiteness may be imparted to the product claim by that part of the specification which purportedly details only a method of making the product, the description of the Pacz process is likewise silent as to the nature of the filament product.

Id. at 373. The Court held the patent invalid for lack of a "distinct and definite" description of the invention, for the court "doubted whether one who discovers or invents a product he knows to be new will ever find it impossible to describe some aspect of its novelty." Id. Whatever the inadequacies in the Pacz description of his invention, the Court's optimistic view of scientific capability cannot be deemed to have

barred all recourse to the rule of necessity when it is warranted, or to have voided the ensuing seventy-one years of Patent Office and judicial recognition of this pragmatic expedient.

No Supreme Court case discussed the problems of complexity and structural analysis that warrant this expedient, or created a legal solution to these problems. It is inappropriate, unsupported by law or precedent, and contrary to the purposes of patent systems, for this court now to rule that such products cannot be patented as products.

Regional circuit decisions

My colleagues also rely on some decisions of the regional circuits preceding this court's formation, announcing that "our sister circuits also followed the general rule that the defining process terms limit product-by-process claims," and citing two cases, one decided in 1915 and one in 1977. These cases do not support the en banc court's opinion,[4] and raised no issue of an expedient based on necessity.

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[4] It is curious to observe this en banc court extolling decisions of the regional circuits as authoritative, while it disregards the decisions of our predecessor courts and of this court. This court was created to remove patent law questions from the regional circuit courts. See H.R. Rep. 96-1300, at 20 (1980) ("Directing patent appeals to the new court will have the beneficial effect of removing these unusually complex, technically difficult, and time-consuming cases from the dockets of the regional courts of appeals. . . . [T]he central purpose is to reduce the widespread lack of uniformity and uncertainty of legal doctrine that exist in the administration of patent law."); see also Federal Courts Improvement Act of 1979: Hearings Before the Subcomm. on Improvements in Judicial Machinery of the Comm. on the Judiciary, U.S. Senate, 96th Cong. 197 (1979) (statement of Hon. Henry J. Friendly) ("What is needed is a group of judges, some but not all patent lawyers, with scientific training and interest, aided both by law clerks of similar bent and by a staff of experts in a variety of technologies, such as the Court of Customs and Patent Appeals has had for years and the courts of appeals in the very nature of things, cannot . . . "). To cite two regional circuit decisions, while jettisoning the precedents of the court uniquely qualified to address patent questions and selected to supplant the regional circuits, is puzzling.

In Hide-Ite Leather Co. v. Fiber Products Co., 226 F. 34 (1st Cir. 1915), the appeal was of two process claims for making leatherboard, and a product claim for leatherboard "made from pulp" and reciting the second step in the process claims plus the reference "substantially as described." The accused infringer did not use the same first step of the process. The court found that the invention was for a process, not a product, and therefore that the product claim was not infringed.

My colleagues also cite Paeco, Inc. v. Applied Moldings, Inc., 562 F.2d 870, 876 (3d Cir. 1977), in which the court used the specification to resolve an ambiguity in the language of a product claim relating to "replica wooden beams" made of foamed urethane. The court reviewed whether ambiguous claim language required a closed or open mold, for this determined the question of anticipation based on a prior art reference that used an open mold. Thus the court stated that the manufacturing process described in the specification was "of paramount importance," and construed the claim in light of that process as requiring a closed mold, thus preserving the claim's validity as against the prior art that used and open mold. The sentence quoted by my colleagues out of its context, does not relate to the en banc court's new rule concerning process terms in product claims, and the Paeco case raised no question of whether the product was capable of description apart from the process.

In addition to these two cases inaptly cited by the en banc court, other regional circuit decisions also contradict this court's new thesis. In Dunn Wire-Cut Lug Brick Co. v. Toronto Fire Clay Co., 259 F. 258 (6th Cir. 1919), the court stated: "Certain it is, in view of the weight of authority and the latest decisions, that the inventor of a new and

useful product or article of manufacture may have a patent which covers it and gives a monopoly upon it regardless of great variations in the method of making." Id. at 261.

In Buono v. Yankee Maid Dress Corp., 77 F.2d 274 (2d Cir. 1935) (L. Hand, J.), the court held invalid a product claim for a kind of "blind stitch" used in sewing, because the invention lay only in the process of producing the stitch, which itself "was not new." Id. at 279. While the stitch had not been claimed as the product of a particular machine or process, the court remarked on the conceivability of patenting such a product "merely as the product of a machine or process, even though it were anticipated if made in other ways," id., observing that such a claim might serve a useful purpose in protecting against products that were produced by the same machine or process abroad and then imported. Of such a claim, wherein the product itself was anticipated but the process was new, the court stated "it would in that case not be infringed by anything but the product of the . . . process." Id. This routine statement of established law does not mean that when the product is itself new and useful and unobvious, it cannot be claimed as a product but must be tied to the machine that made it.

Judge Hand emphasized that this example related only to situations where the product itself was not new. The opinion explained that to be claimed as a product, the product "must be new as such, that is, regardless of the process or machine which makes it; and it must stand upon its own invention, again independently of the machine or process which makes it." Id. This was also the CCPA's view of product claims, providing the precedent carried forward to, and binding upon the Federal Circuit.[5]

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[5] There has been extensive commentary on this class of claim. See, e.g., Jon S. Saxe & Julian S. Levitt, Product-by-Process Claims and Their Current Status in Chemical Patent Office Practice, 42 J. Pat. Off. Soc'y 528, 559 (1960) ("Except in the

III

THE EN BANC RULING

Defying precedent, the en banc court adopts for all situations "the basic rule that the process terms limit product-by-process claims," maj. op. at 17, whether the product is novel or known, and whether or not the new product could not have been fully described by its structure alone. The court eliminates the long-accepted expedient for new products whose structure is not fully known. While the Scripps decision is the only decision that is mentioned as "expressly overruled," maj. op. at 17, Scripps is only one of many cases now discarded.

The en banc majority's response to the dissenters is to state that "the inventor is absolutely free to use process steps to define this product" if its "structure is either not fully known or too complex to analyze," maj. op. at 19, but to eliminate the premise that the inventor thereby obtains a product claim, not a process claim. According to the majority, a patentee can continue to obtain product claims using process descriptors, but such product claims are treated as process claims for infringement. The applicant would still have to demonstrate patentability of the new product as a product (independent of the process), while enforcement of the patent against an identical product would be limited to the infringer's use of the process steps used as a descriptor. For the first time, claims are construed differently for validity and for infringement.

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chemical arts, a claim to a product must be in terms of the product's objective physical and chemical characteristics; but where these are unknown or impossible to express, a claim may define a product in terms of the process by which it is made. This product-by-process exception is to be distinguished from the use of process terminology as descriptive of a state of being."); Brian S. Tomko, Scripps or Atlantic: The Federal Circuit Squares Off Over the Scope of Product-by-Process Patents, 60 Brook. L. Rev. 1693, 1696 (1995) (the Atlantic decision "pared the scope of a product-by-process patent to that of a glorified process patent").

It has been an inviolate rule that patent claims are construed the same way for validity and for infringement. See, e.g., Amgen Inc. v. Hoechst Marion Roussel, Inc., 324 F.3d 1313, 1330 (Fed. Cir. 2003) ("It is axiomatic that claims are construed the same way for both invalidity and infringement."); Amazon.com, Inc. v. Barnesandnoble.com, Inc., 239 F.3d 1343, 1351 (Fed. Cir. 2001) ("Because the claims of a patent measure the invention at issue, the claims must be interpreted and given the same meaning for purposes of both validity and infringement analyses."); C.R. Bard, Inc. v. M3 Systems, Inc., 157 F.3d 1340, 1363 (Fed. Cir. 1998) ("Claims must be interpreted the same way for determining infringement as was done to sustain their validity."); Southwall Technologies, Inc. v. Cardinal IG Co., 54 F.3d 1570, 1576 (Fed. Cir. 1995) ("Claims may not be construed one way in order to obtain their allowance and in a different way against accused infringers."); Beachcombers, International, Inc. v. WildeWood Creative Products, Inc., 31 F.3d 1154, 1163 (Fed. Cir. 1994) ("We have already interpreted the claims for purposes of assessing their validity. The same claim interpretation of course applies to the infringement analysis."); Scripps Clinic & Research Foundation v. Genentech, Inc., 927 F.2d 1565, 1583 (Fed. Cir. 1991) ("claims must be construed the same way for validity and for infringement"); Smithkline Diagnostics, Inc. v. Helena Laboratories Corp., 859 F.2d 878, 882 (Fed. Cir. 1988) ("The claims of the '970 patent measure the invention at issue; thus, the claims must be interpreted and given the same meaning for purposes of both validity and infringement analyses."); see also 5A Chisum on Patents §18.01 (2007) ("A fundamental tenet of patent law is that a claim must be interpreted consistently for purposes of infringement and validity."); id. §18.03[2][h] (collecting cases).

As interpreted for validity, the claims obtained under the expedient of necessity are product claims, and are subject to the requirements of novelty, unobviousness, and all other requirements for new products, independent of how the products can be made. My colleagues hold that these are product claims for validity, but process claims for infringement. Departure from the rule that forbids such deviation requires sound reason, and fuller exploration than the cursory brush-off dispensed by my colleagues.

I do agree with my colleagues that their logic is "simple." Maj. op. at 19. However, today's inventions are not simple. The needs of inventions of the past and present, and more so the future, are not simple. The public interest in invention and development of today's complex sciences, is not simple. The en banc court's "simple" hypothetical about "compound X, obtained by process Y," is simply irrelevant to the issues we must resolve. Scientists know that it is often easier to show that two products are the same, than to decipher their chemical or biological structure; for example, in the case at bar, comparing the X-ray diffraction patterns and absorption spectra could show that the products are the same, although their exact crystal structure is undefined. However, my colleagues announce that the only way to establish whether the accused compound is the same as the patented compound is by inquiring whether they were prepared by the same method. Maj. op. at 19-20 ("[W]hat analytical tools can confirm that the alleged infringer's compound is in fact infringing, other than a comparison of the claimed and accused infringing processes?"). That question has many answers, now stated to be irrelevant.

While the section of this opinion decided by the en banc court is largely directed to its reversal of precedent, the implementation of its ruling remains with the original

panel. The panel decision enlarges the en banc ruling, further binding this court. The claims at issue state processes by which the new crystal form is "obtainable," although the specification states that other methods might be used. The panel rules that a claim "cannot capture a product obtained by or obtainable by processes other than those explicitly recited in the claims." maj. op. at 21, finding authority in BASF, which I have discussed ante. My colleagues thus continue to misapply the Court's ruling in BASF, where the Court stated repeatedly that the product in that case was a known product. BASF, 111 U.S. at 311 ("It was an old article."). In BASF the Court responded to the patentee's argument that it was entitled to cover all artificial alizarine made by any process, by observing that the patentee had not shown how the infringing and patented products "can be recognized," id. at 310, an aspect at the opposite pole from the case at bar, where the patentee provided elaborate details as to how the patented and accused crystal forms can be recognized.

The panel also states that "the applicant's statement in the file wrapper that 'the method of preparation . . . is not considered the heart of the present invention' should not be afforded undue gravitas." Maj. op. at 22. This too is an aberration of precedent, and is contrary to the many rulings of the Supreme Court and this court that afford due gravitas to the applicant's statement of what has been invented. See, e.g., BASF, 111 U.S. at 308 ("It is very plain that the specification of the original patent, No. 95,465, states the invention to be a process for preparing alizarine, not as a new substance prepared for the first time, but as the substance already known as alizarine, to be prepared, however, by the new process, which process is to be the subject of the patent . . . ."); Plummer v. Sargent, 120 U.S. at 443 (quoting specification of companion

patent, where inventor stated "My invention consists in a process of covering iron with a very thin coating of oil, and then subjecting it to heat, the effect of which is to leave upon the iron a firm film, which is very durable, and gives the iron a highly ornamental appearance, like that of bronze"). The Federal Circuit's emphasis on the importance of the specification has been repeatedly stated. E.g., Phillips v. AWH Corp., 415 F.3d 1303, 1315 (Fed. Cir. 2005) (en banc) ("[T]he specification is always highly relevant to the claim construction analysis. Usually, it is dispositive; it is the single best guide to the meaning of a disputed term." (internal quotation marks omitted)).

The en banc court appears to misjudge the implications of its ruling, for the court states that it is now making available to "others the right to freely practice process Z [a different process] that may produce a better product in a better way." Maj. op. at 20. If others can indeed make a better product, this expedient presents no impediment. That is not the issue of this case. The issue is the right to make the same product, by making a process change that does not change the product. By now assuring that right, the exclusionary value of the claim to a new product is lost.

The purpose of the rule of necessity is to allow inventors of complex new products to obtain the patent scope to which their invention is entitled—the scope of the novel product they invented, no more and no less. The majority's change of law simply imposes unfairness as well as legal error on patent-supported advances.

SUMMARY

Precedent establishes that the correct construction of claims that recite process steps depends, like all claim construction, on what has been invented. No single rule fits all inventions. The construer must view the claims in light of the description of the

invention in the specification, the prior art, and the prosecution history. In the complex law and practice of patents and inventions, the special expedient here of concern arises when the precise structure of a new product is not known from the information available when the patent application was filed. The law has enabled and endorsed this expedient of describing a product in order to claim it as a product, whereby validity and infringement are determined as a product, independent of any process term that was used to aid in defining the product. This expedient does not enlarge patent scope; it simply permits patenting what has been invented. A narrow but clear body of law has evolved to accommodate this need of complex technologies. This entire body of law is today overturned, sua sponte and without a hearing, without any participation of those affected, without identification of the intended benefits. I respectfully dissent from the en banc court's rulings, as well as the procedure by which they were reached.

United States Court of Appeals for the Federal Circuit

2007-1400

ABBOTT LABORATORIES,

Plaintiff-Appellant,

and

ASTELLAS PHARMA, INC.,

Plaintiff-Appellant,

v.

SANDOZ, INC.,

Defendant-Appellee,

and

SANDOZ GMBH,

Defendant,

and

TEVA PHARMACEUTICALS USA, INC. and
TEVA PHARMACEUTICAL INDUSTRIES, LTD.,

Defendants-Appellees,

and

RANBAXY LABORATORIES, LTD. and RANBAXY, INC.,

Defendants,

and

PAR PHARMACEUTICAL COMPANIES, INC. and PAR PHARMACEUTICAL,

Defendants.

2007-1446

LUPIN LIMITED,

Plaintiff/Counterclaim Defendant-Appellee,

and

LUPIN PHARMACEUTICALS, INC.,

Counterclaim Defendant-Appellee,

v.

ABBOTT LABORATORIES,

Defendant/Counterclaimant-Appellant,

and

ASTELLAS PHARMA, INC.,

Defendant/Counterclaimant-Appellant.

Appeals from the United States District Court for the Northern District of Illinois in case no. 07-CV-1721, Judge Wayne R. Andersen and the United States District Court for the Eastern District of Virginia in case no 3:06-CV-400, Judge Robert E. Payne.

LOURIE, Circuit Judge, dissenting from en banc Section III. A. 2.

I respectfully dissent from the court's en banc holding in Section III. A. 2 that product-by-process claims always require use of the recited process in order to be infringed.

I agree that there is substantial Supreme Court precedent that holds that product-by-process claims require use of the recited process for there to be infringement. However, many of those cases applied overly broad language to fact situations involving old products or used vague language that makes it difficult to determine

whether the products were old or new. Clearly, however, when a product is old, a product-by-process claim cannot be interpreted as a claim to the product made by any means. The product is old and unpatentable per se. BASF in fact involved an old product. See Cochrane v. Badische Anilin & Soda Fabrik, 111 U.S. 293, 311 (1884) ("It was an old article.").

There is arguably a different situation that should apply to chemical-biological products today than to mechanical products of more than a century ago. When a product is new and the inventor claims it by a process of preparation, I fail to see why the product-by-process claim should not be interpreted as a product claim that can be infringed even when the product is made by means other than that recited in the claim. Supreme Court precedent dealing with old products, while utilizing broad language, does not foreclose that possibility. The Court years ago did not have occasion to consider today's innovations or decide whether a distinction should be made between a new chemical-biological product and an old product made by a new process.

And there may be differing results depending upon the exact wording of a claim at issue. For example, a claim reading "when made by" might only be infringed when the recited process is used by the accused, as it is situational. On the other hand, a claim reading "obtainable by" refers to capability, so it might not require use of the process to infringe. "Obtained by" is ambiguous. Bright lines have their uses, but judging should take account of differing circumstances. In addition, of course, in order to sustain any claim for infringement, a patent owner must prove that an accused product is the same as that covered by an asserted claim. If the reason a product was claimed by its process was that its structure was unknown, then, if, at the time

infringement is asserted, there still is no means to ascertain structurally whether the accused product is the same as that claimed, the infringement claim fails. However, that should not mean that a new product claimed by a process of preparation cannot ever be infringed when made by another process.

It may be that with today's analytical techniques there is little need for product-by-process claims. After all, claim 1 of the Abbott patent is a claim to a compound, not only by name, but also by certain of its characteristics. A claim to a product defined by its characteristics or properties surely is a proper claim.

However, product-by-process issues still seem to come before us and I would make a distinction between old products and new products in interpreting product-by-process claims. Accordingly, I respectfully dissent from the court's en banc holding.

EXHIBIT B

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Different Biological Characteristics of Wild-Type Porcine Reproductive and Respiratory Syndrome Viruses and Vaccine Viruses and Identification of the Corresponding Genetic Determinants[▽]

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Two attenuated vaccines, Ingelvac PRRS MLV and Ingelvac PRRS ATP, derived from VR2332 and JA142, respectively, have been used to control porcine reproductive and respiratory syndrome (PRRS) virus. However, there have been several field reports concerning the reversion of the vaccine virus to virulence. Furthermore, viruses genetically indistinguishable from the vaccines and wild-type parental viruses have been detected in clinical PRRS cases, raising the need for a better differential tool. As the vaccine viruses replicated better and produced bigger plaques in MARC-145 cells than did the wild-type parental strains, the following study was conducted to determine if the growth difference in MARC-145 cells can be utilized to differentiate a vaccine-like virus (VLV) from a wild-type virus and to identify genetic markers corresponding to such phenotype of the vaccine viruses. The relatedness of 83 field isolates collected between 1996 and 2005 to VR2332 and JA142 was classified genetically and antigenically. Thirteen of 25 VR2332-related viruses and 9 of 10 JA142-related viruses were determined as VLVs, since those viruses produced plaques similar to those by the vaccine viruses. Four unique amino acids each were identified throughout structural genes for MLV and ATP. Among those, F^{10} in open reading frame 2 (ORF2) of MLV and E^{85} and Y^{165} in ORF3 of ATP were stable during pig passages. When the sequences unique for MLV were incorporated into an infectious clone constructed based on VR2332, the virus growth and resultant plaque size in MARC-145 cells were increased, suggesting that these sequences can be used as genetic markers for VLVs.

Porcine reproductive and respiratory syndrome (PRRS) is characterized by reproductive failure in breeding animals and respiratory distress in all ages of pigs (1, 3). The syndrome was first reported in the United States in 1987 (11). The causative agent of PRRS was, however, isolated first in The Netherlands in 1991 and named the Lelystad virus, the European prototype PRRS virus (PRRSV). Isolation of VR2332, the North American prototype PRRSV, was subsequently reported in 1992 (3). North American PRRSV (type II) and European PRRSV (type I) share less than 70% sequence homology and comprise two distinct genotypes and serotypes of PRRSV (6).

PRRSV is a small, enveloped virus and belongs to the family *Arteriviridae* in the order *Nidovirales*. PRRS virion contains a positive, single-strand RNA genome of approximately 15 kb in length. The genome encodes at least nine open reading frames (ORFs) (2, 7). ORF1a and -1b encode nonstructural proteins required for virus replication (2). ORF2a, -3, and -4 encode three *N*-glycosylated minor envelope proteins designated GP2, -3, and -4, respectively, while ORF2b, which is completely embedded in ORF2a, encodes a nonglycosylated minor envelope protein named 2b, which is equivalent to E protein of equine arteritis virus (24). ORF5, -6, and -7 encode the major envelope (GP5 or E), membrane (M), and nucleocapsid (N) proteins, respectively (7).

PRRS has been identified worldwide and caused a significant economic loss to the swine industry. The economic impact of PRRS to U.S. swine producers has been estimated at an approximate $560 million loss per year (19). In response to the economic significance of PRRS, two attenuated live-virus vaccines (Ingelvac PRRS MLV and Ingelvac PRRS ATP) have been used most to control the disease (12). Ingelvac PRRS MLV (hereafter "MLV") vaccine, which was derived from VR2332 after sequential passage of the virus in a monkey kidney cell line (CL2621), was first introduced to the U.S. market in 1996 (16). This vaccine has been demonstrated to reduce the rate of PRRSV-associated reproductive failure in sows and lessen clinical symptoms in younger pigs (8, 18). Nonetheless, a severe form of PRRS (a.k.a. acute or atypical PRRS), which was described as "abortion storm" or "sow mortality syndrome," has been reported for MLV-vaccinated farms. The incidence of this acute form of disease in vaccinated pigs resulted in the introduction of the Ingelvac PRRS ATP (hereafter "ATP") vaccine, which is made of an attenuated derivative of JA142, to the market in 2000 (12). The JA142 strain was initially isolated from a severe abortion storm case in Iowa in 1997 (17).

Since the vaccines use live viruses even though attenuated, the reversion of vaccine virus to virulence has been a concern and was documented by Danish pig producers after the MLV vaccine was introduced to Denmark in 1995 as part of the national PRRS control program. After the introduction, viruses closely related to the MLV vaccine virus were isolated from clinically ill pigs in 1996 (16). Since no North American

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PRRSV was reported in Denmark or other European countries until the spring of 1996, it was concluded that these field isolates originated from the vaccine virus (16, 25). Unlike the Danish experience, the differentiation of some field isolates of PRRSV from wild-type parental and vaccine viruses has been challenging in the U.S (21) due to the antigenic and genetic similarity between the vaccine and wild-type parental viruses (25). A few molecular assays, such as sequencing or restriction fragment polymorphism analysis, have been extensively employed for the differential purpose (28). However, a great number of field isolates which are genetically indistinguishable from both a vaccine virus and its parental strain have been continuously identified from clinical PRRS cases (29), raising the need for a better way to differentiate wild-type viruses from the vaccine viruses.

Due to the fact that the vaccine virus was attenuated by sequential passages in cell culture, the vaccine viruses tend to grow much better in MARC-145 cells, a highly permissive clone of African monkey kidney cell line MA104 (13), than in their wild-type parental viruses (14). Recently it was also observed in our laboratory that the vaccine virus produced a significantly bigger-sized plaque in MARC-145 cells than what was seen for their parental strains. The following study was to evaluate if the difference in growth characteristics (i.e., phenotype) between the vaccine strains and their wild-type parental viruses in MARC-145 can be utilized to aid strain differentiation by compensating for the limitation of genetic differentiation. In addition, attempts were made to identify sequence elements associated with the phenotype of the vaccine or related viruses (i.e., the attenuated form of PRRSV). Furthermore, the utility of such sequence elements as surrogate markers for the rapid detection of vaccine-like viruses (VLVs) was assessed.

MATERIALS AND METHODS

Study design. Field isolates (n = 83) of PRRSV were classified into the VR2332 group, the JA142 group, and an unrelated wild-type virus group based on their genetic (i.e., sequencing) and/or antigenic (i.e., one-way cross virus neutralization [VN]) similarities. Then, a plaque assay was applied to the isolates in order to determine how well the virus grows (i.e., the highest progeny virus titer and time to peak titer) and the size of plaque produced in MARC-145 cells compared to those of the vaccine and their wild-type parental viruses. The stability of the phenotypic characteristics of the wild-type and vaccine viruses were assessed by sequentially passing the viruses in cell culture and animals, respectively. In addition, structural genes (ORF2 to -7) of vaccine viruses or VLVs and wild-type viruses were compared to identify sequence elements for the vaccine viruses or VLVs. A reverse genetics system constructed based on the sequence of VR2332 (20) was then employed to determine the biological role of the identified genetic markers unique for MLV.

Viruses and cells. Eighty-three field isolates of PRRSV which were collected from submissions to Iowa State University Veterinary Diagnostic Laboratory during the period from 1999 to 2005 were employed for the study. Besides the field isolates, two vaccine viruses (MLV and ATP) and their parental strains (VR2332 and JA142, respectively) were used as reference viruses. The vaccine viruses were kindly provided by Boehringer-Ingelheim Vetmedica, Inc., in Ames, IA (14). All field and reference viruses were propagated in MARC-145. All viruses represented five passages in the cell.

Characterization of growth of reference viruses in MARC-145 cells. To determine the one-step growth of VR2332 and MLV, confluent monolayers of MARC-145 prepared in 25-cm^2 flasks and 48-well plates were inoculated with VR2332 or MLV at a multiplicity of infection of 0.01. After a 1-hour incubation at 37°C, the inoculums were discarded and the cells were replenished with RPMI-1640 medium (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (Sigma), 20 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamicin, and 0.25 μg/ml amphotericin B (hereafter "RPMI growth medium"). Every 3 h postinoculation (p.i.), cell culture fluid and cells were collected separately from each flask for 24 h. The virus titer (50% tissue culture infective dose [$TCID_{50}$]/0.1 ml) in each of the cell culture fluids or cell lysate supernatants was determined by a microtitration infectivity assay (9, 23) in MARC-145 cells. At each collection time, cells in the corresponding 48-well plate were simultaneously fixed with cold 80% acetone aqueous solution for a fluorescent antibody test to assess the production of viral proteins.

Growths of the VR2332, MLV, JA142, and ATP strains were also assessed for a multistep growth curve. Each of the viruses was inoculated onto confluent monolayers of MARC-145 prepared in 25-cm^2 flasks at a multiplicity of infection of 0.01. After a 1-hour incubation at 37°C, the inoculum was discarded and the cells were replenished with RPMI growth medium. One flask of MARC-145 cells inoculated with each strain was frozen at 6, 12, and 24 h and thereafter every 24 h until 4 days p.i. After three rounds of freeze-thawing at −80°C and 37°C, respectively, the cell culture fluid was harvested for virus titration.

Plaque assay. The assay was performed as previously described (22) with some modifications. One hundred or 10 fluorescence focus-forming units (FFUs) of each virus in 0.1 ml of RPMI growth medium were inoculated on confluent monolayers of MARC-145 prepared in 24-well plates. The cells were incubated for 1 h at 37°C and the inoculum was replaced with overlay medium (RPMI growth medium containing 0.6% of agar). The cells were further incubated for up to 4 days at 37°C until plaques were clearly observed. The overlay medium was removed from each well of the plates and cell monolayers were stained for 30 min with 0.25% Coomassie brilliant blue R250 staining solution (Bio-Rad, Hercules, CA), which was prepared in the mixed solution of absolute acetic acid and 50% methanol at a ratio of 1:9 (vol/vol). The diameter (mm) of plaque produced by each virus was determined by averaging the sizes of the three well-defined plaques observed. A plaque bigger than 3 mm in diameter was considered as a big-sized plaque, and one smaller than 2 mm in diameter was considered as a small-sized plaque. A plaque between 2 and 3 mm in diameter was regarded as a medium-sized plaque. The assay was repeated at least three times for all viruses examined.

VN assay. A florescent focus neutralization assay was conducted as previously described (30) with some modifications to assess the susceptibility of field PRRSV isolates to the neutralizing activity of antisera produced in pigs against the VR2332 and JA142 strains. The antisera had VN titers of 1:64 and 1:32 against the homologous virus, respectively. Sera collected from PRRSV-naïve age-matched pigs served as a negative control. A pair of anti-PRRSV antibody-positive and -negative sera was serially diluted twofold in 100 μl of RPMI growth medium and mixed with an equal volume of each virus at a rate of 100 FFU/μl. Mixtures of virus and serum were then incubated for 1 h at 37°C. Each mixture was inoculated onto MARC-145 cell monolayers prepared in 96-well plates and incubated for another hour at 37°C. The plates were then replenished with 100 μl of fresh RPMI growth medium per well and further incubated for 20 h at 37°C in a humid 5% CO_2 atmosphere. After the incubation, the cells were fixed with cold 80% acetone aqueous solution for 2 min at ambient temperature and incubated with PRRSV-specific monoclonal antibody SDOW-17 conjugated with fluorescent isothiocyanate (Rural Technologies, Brookings, SD). After a 1-h incubation, the cells were washed three times with phosphate-buffered saline, and the number of virus-specific fluorescent foci in each well was counted. VN titer was expressed as the reciprocal of the highest dilution in which a 90% or higher reduction in the number of FFU was observed.

Sequencing and sequence analysis. All 83 field isolates were sequenced for ORF2, -3, -5, and -6, and selected viruses (n = 50) were sequenced for ORF2 to -7. PCR and sequencing primers were designed as follows: ORF2 to 6 Forward (P5F), 5′-CCACTGCCACCAGCTTGAAGTT-3′; ORF2 to 6 Reverse (P5R), 5′-CAGA CACAATTGCCGCTCACTAGG-3′; ORF2 Forward (P2F), 5′-AAACGGTGAG GACTGGGAGGATTA-3′; ORF2 Reverse (P2R), 5′-TCGAAAGAAAAATTGC CCCTAACC-3′; ORF3 Forward (P3F), 5′-CCGGTTGGCTGGTGGTCT-3′; ORF3 Reverse (P3R), 5′-CAAAACAGAACGGCACGATACACC-3′; ORF5 Forward (P5F), 5′-CCTGAGACCATGAGGTGGG-3′; ORF5 Reverse (P5R), 5′-TT TAGGGCATATATCATCACTGG-3′; ORF6 Forward (P6F), 5′-GCGGTCGCC TGTCATCATAG-3′; ORF6 Reverse (P6R), 5′-GGCTGGCCATCCCCCTTCTT TCT-3′; ORF7 Forward (P7F), 5′-TCGTGTTGGGTGGCAGAAAAGC-3′; and ORF7 Reverse (P7R), 5′-GCCATTCACCACACATTCTTCC-3′.

Viral RNA was extracted using the QIAamp viral RNA mini kit (Qiagen, Valencia, CA). For sequencing the full length of ORF2 to -6, reverse transcription (RT) was conducted using Superscript III (Invitrogen, Grand Island, NY) according to the manufacturer's protocol, and cDNA was amplified with the GeneAmp XL long PCR kit (Applied Biosystems, Foster City, CA). The cycling conditions were 94°C for 1 min, 16 cycles of 94°C for 15 s, and 68°C for 4 min followed by 14 cycles of 94°C for 15 s and 68°C for 4 min, with time increased by 15 s per cycle, and then final extension at 68°C for 10 min. To sequence individual ORFs, the target ORF was directly amplified with the Qiagen one-step RT-PCR

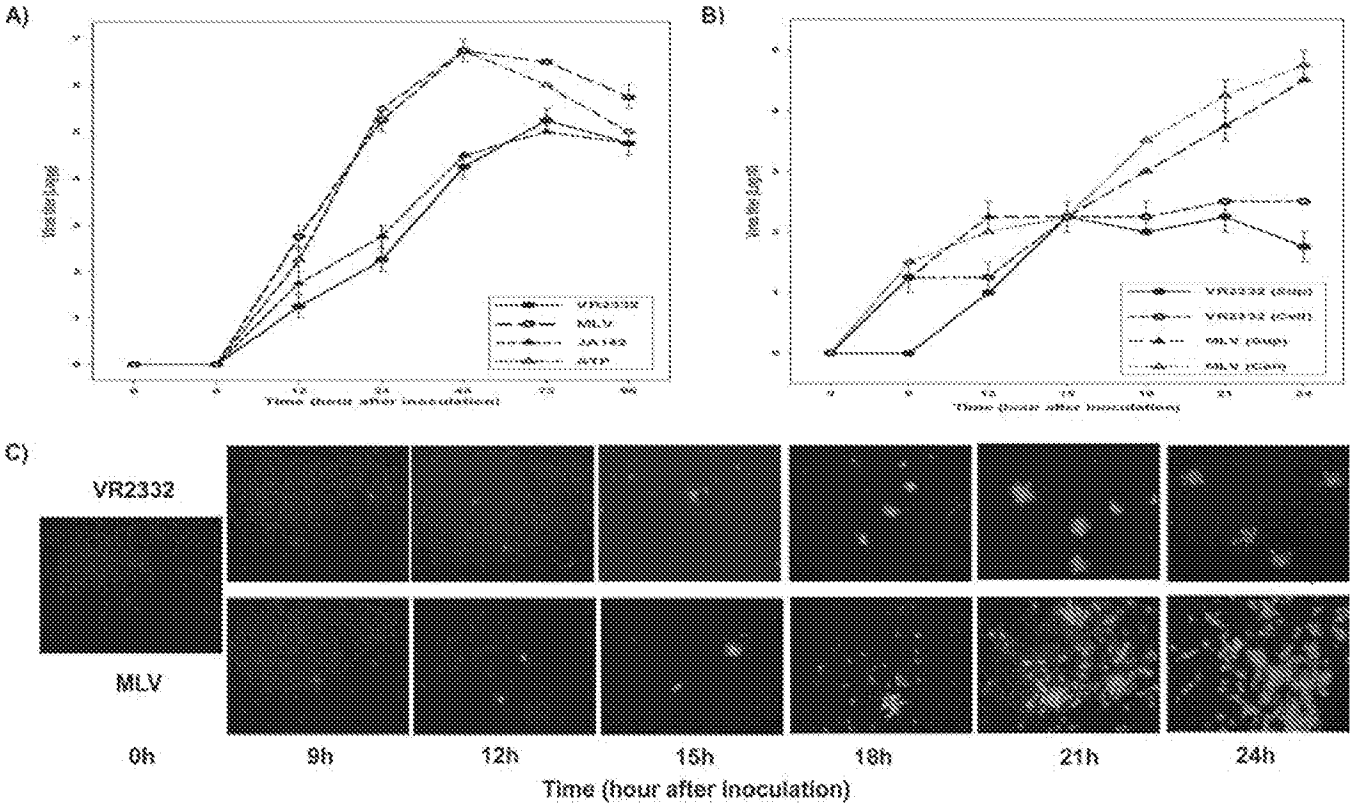

FIG. 1. Different growth characteristics of wild-type PRRSVs (VR2332, JA142) and their cell-attenuated vaccine viruses (MLV, ATP) in MARC-145 cells as measured by multistep growth (A) and one-step (B) curves and immunofluorescence microscopy (C). Sup, supernatant.

kit (Qiagen). The reaction conditions were 50°C for 30 min for RT and 95°C for 15 min followed by 30 cycles of 95°C for 30 s, 50°C for 45 s, and 72°C for 1 min; final extension was conducted at 72°C for 10 min.

All PCR products were purified using the QIAquick PCR purification kit (Qiagen) and submitted to the Iowa State University Nucleic Acid Facility for sequencing. Once sequence data were available, multiple alignments and sequence comparisons were conducted using Lasergene software (DNASTAR Inc., Madison, WI). Unrooted dendrograms were generated by the distance-based neighbor-joining method using Molecular Evolutionary Genetic Analysis (MEGA) software (15). Bootstrap values were calculated on 1,000 replicates of the alignment to determine the reliability of the phylogram.

Assessment of the stability of phenotypic characteristics. The stability of cell growth phenotypes (i.e., attenuated versus wild) of PRRSVs was determined in vitro and in vivo. For an in vitro assessment, the VR2332 and JA142 strains were sequentially passed in MARC-145 cells for 20 passages. Sequential passages were repeated three different times for each virus, resulting in a total of 120 viruses derived from the two strains for plaque assay and sequence analysis.

For an in vivo assessment, the CC-01 strain, a plaque-cloned virus derived from VR2332 through cell passages, was sequentially passed in pigs housed in HEPA-filtered isolation units for 13 times. The CC-01 strain shared high sequence homology with MLV (99.6% for entire structural genes) and showed phenotypic characteristics similar to those for MLV virus in cells and animals. It produced large plaques (>4 mm) similar to those seen for the vaccine viruses and induced a low level of viremia with minimum virulence in the inoculated pigs. Details of the pig-to-pig passage study were reported elsewhere (6). In brief, at each passage, a total of three pigs were used for inoculation of the virus material, and the fourth pig served as a sham-inoculated control. At the completion of the 60-day observation period in each passage, all pigs were euthanized and a variety of tissues was collected. Homogenates of mixed tissues were used to inoculate pigs in the subsequent passage in a manner to keep three independent lines of pig passage (A, B, and C). After seven passages, line A was discontinued. Two of 15 plaque-cloned viruses isolated from each pig's serum collected at day 7 p.i. in each passage were used for plaque assay and sequence analysis. A total of 66 plaque-cloned isolates (three pigs/passage times 2 isolates/pig times seven passages plus two pigs/passage times 2 isolates/pig times six passages) were examined.

Generation of mutant virus. The cDNA infectious clone constructed based on the sequence of VR2332 was used for this study (20). Mutagenesis was performed using the QuikChange II site-directed mutagenesis kit (Stratagene, West Cedar Creek, TX) as per the manufacturer's manual. A shuttle vector (TOPO XL PCR cloning vector; Invitrogen, Carlsbad, CA) containing the whole structural genes (ORF2 to -7) of VR2332 was constructed and used as a template for mutagenesis to prevent any unexpected mutations during the process. The targeted mutation was confirmed by sequencing the whole structural genes in the shuttle vector, and then the whole fragment of the structural genes in the shuttle vector was substituted for those genes in the plasmid of the infectious clone by use of BsrGI and HpaI sites. The infectious clone containing only a targeted mutation in the right position was selected by sequencing the entire structural genes of the plasmid purified from competent cells (XL10-Gold ultracompetent cells; Stratagene). The plasmid of mutant infectious clone was then linearized by AclI and purified using DNAclear kit (Ambion, Austin, TX). The linearized plasmid was transcribed using T7 promoter by the mMESSAGE mMACHINE T7 kit (Ambion). The transcribed RNA was purified by the MEGAclear kit (Ambion) and 1 to 10 μg of purified RNA was transfected into MARC-145 cells (5×10^6 cells/ml) prepared in chilled Dulbecco's modified Eagle's medium (Sigma) containing 1.25% dimethyl sulfoxide (Sigma) by electroporation at 250 V and 950 μF (Gene Pulser Xcell electroporation system; Bio-Rad) (27). The transfected cells were plated on a six-well plate and the medium was changed with RPMI growth medium 16 to 18 h after plating. At 48 h, 200 μl of the supernatant was inoculated on MARC-145 cells prepared in a 24-well plate. After incubation for 1 h at 37°C, the inoculum was replaced with RPMI growth medium and the cells were further incubated at 37°C until a cytopathic effect became evident. Rescued mutants were grown once in 25-cm^2 flasks and stored at −80°C until used. An aliquot of each mutant was sequenced to confirm the introduction of mutations and the integrity of other sequences.

Data analysis and sequence comparison. The repeated measurements of virus growth were analyzed with multivariate analysis of variance to define the growth difference among the mutants by use of JMP (SAS Institute Inc., Cary, NC).

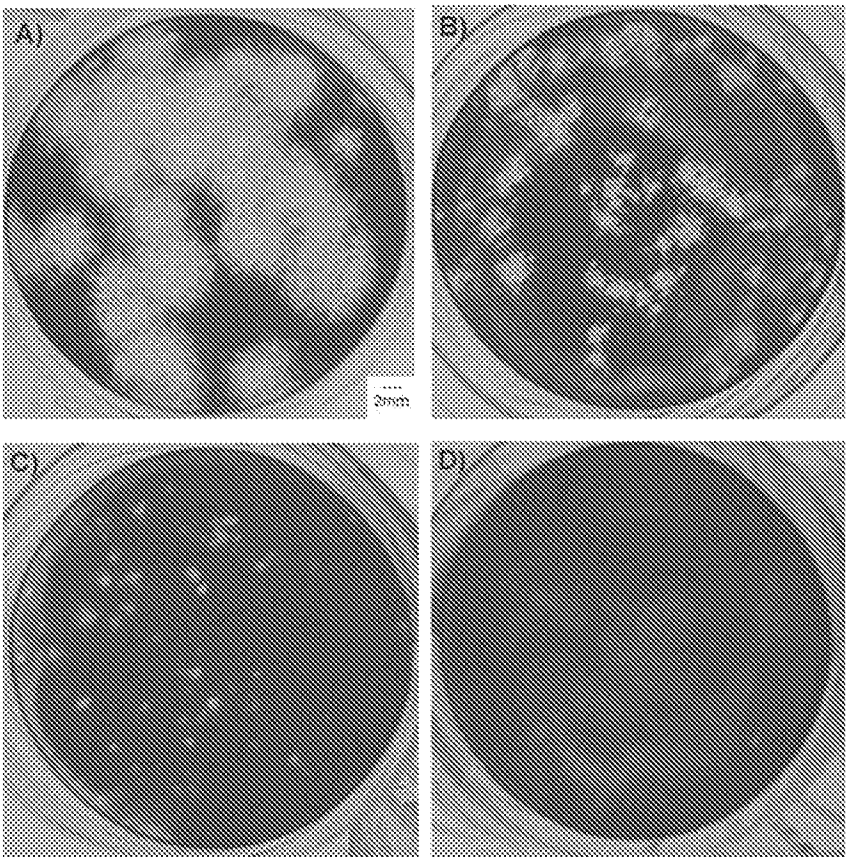

FIG. 2. Photomicroscopy of representative plaques produced by wild-type and attenuated PRRSVs in MARC-145 cells. The vaccine strains or VLVs produce bigger plaques (≥2 mm in diameter) (MLV [A] and 64955-01 [B]), whereas wild-type viruses including the parental strains of the vaccine viruses produce small-sized plaques (<1 mm in diameter) (JA142 [C] and VR2332 [D]).

Sequences were aligned and compared using Lasergene MegAlign software (DNASTAR Inc., Madison, WI).

RESULTS

Phenotypic characteristic of wild-type and vaccine viruses in MARC-145. The vaccine strains (MLV and ATP) grew more efficiently than the wild-type parental viruses (VR2332 and JA142) in MARC-145 cells (Fig. 1A). The progeny virus titers of all four viruses were similar up to 12 h after inoculation. After 12 h p.i., MLV and ATP started to replicate much faster than their wild-type parental strains as the vaccine viruses produced a higher titer of progeny viruses. At 48 h p.i., the progeny virus titer from cells inoculated with the vaccine viruses reached $10^{6.5}$ $TCID_{50}$/0.1 ml, whereas the progeny virus titer from cells inoculated with the wild-type parental strains was approximately $10^{4.5}$ $TCID_{50}$/0.1 ml ($P < 0.001$).

Similar results were observed in the one-step growth curve and immunofluorescence assessment of virus replication. The infectious progeny virus titers of VR2332 and MLV in both the culture fluid fraction (i.e., cell-free form) and the cell fraction (cell-associated form) were similar for each virus during the observation period. However, the kinetics of progeny virus production were significantly different between the two viruses. The MLV virus grew rapidly and steadily to 10^5 $TCID_{50}$/0.1 ml during 24 h p.i., forming a linear growth curve overall, whereas the VR2332 virus grew slowly and reached a plateau (5×10^2 $TCID_{50}$/0.1 ml) after 15 h p.i. (Fig. 1B). In immunofluorescence microscopy, the sizes and numbers of foci produced by VR2332 and MLV were similar until 15 h, but those by MLV increased drastically by 24 h compared to what was seen for VR2332 (Fig. 1C).

The different growth characteristics of the vaccine viruses and their wild-type parental strains yielded the production of differently sized plaques in MARC-145 cells (Fig. 2). The vaccine strains, i.e., MLV and ATP (Fig. 2A), produced larger plaques (diameter, >4 mm on average), while some field strains (Fig. 2B) generated medium-sized plaques (diameter, between 2 and 3 mm on average). In contrast, the parental viruses, i.e., JA142 (Fig. 2C) and VR2332 (Fig. 2D), produced much smaller plaques (diameter, <1 ml on average) within the same incubation time (4 days) and under the same propagation conditions.

Stability of the cell growth phenotypic characteristic of virus. When the CC-01 strain was sequentially passed in three independent lines of pigs 13 times (a total of 726 days of in vivo replication), the faster growth and higher level of viremia began to be observed at the second passage of the virus (4). In contrast to the CC-01 virus that produced large-sized plaques (diameter, >4 mm) in MARC-145, its descendants collected from subsequent pig passages started to produce plaques

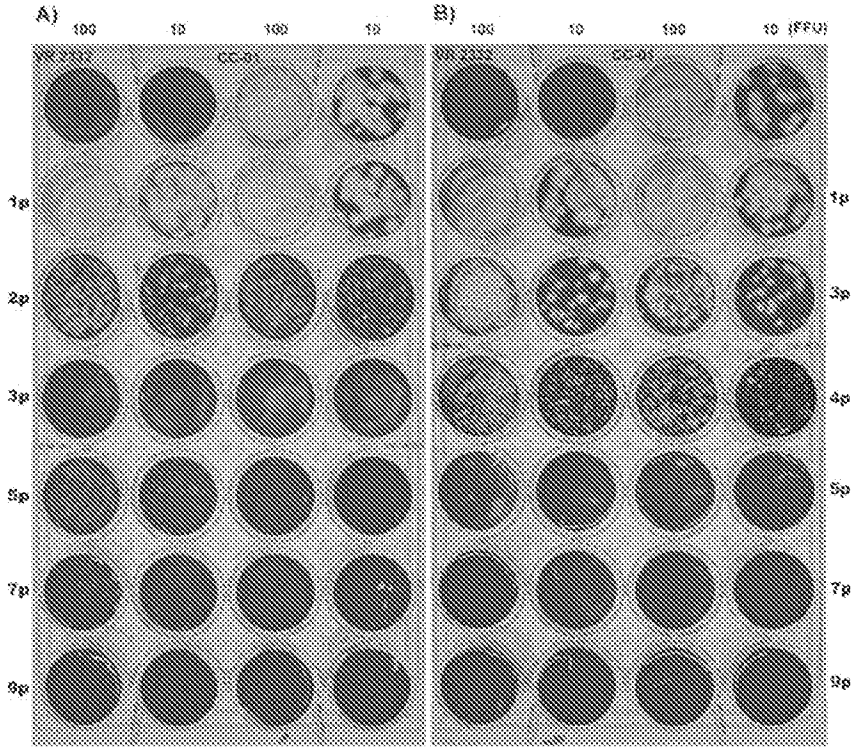

FIG. 3. The change in the size of plaques produced by a MLV vaccine-like PRRSVs (CC-01) during sequential pig-to-pig passages which were maintained in three independent lines (A, B, and C). Two plaque-cloned viruses isolated from each serum sample collected 7 days p.i. at each passage were used for the plaque assay. (A and B) Viruses collected from pig line B (results for line A were identical) and line C, respectively, during the passages. Numbers on top indicate the amounts of virus inoculated to MARC-145 cells (duplicate wells/virus) as expressed in FFU per 0.1 ml. Numbers with suffix "p" represent the number of pig passages of the CC-01 strain. The viruses after 9 passages (9p) consistently produced small-sized plaques.

smaller than those by the CC-01 after two or three pig passages (Fig. 3). After the third (Fig. 3, lines A and B) or fourth (Fig. 3, line C) passage, all recovered progeny viruses produced plaques with diameters of less than 2 mm. Once the plaque size became small, the viruses recovered from subsequent pig passages produced the same small-sized plaques until the termination of pig-to-pig passages.

During sequential cell culture passages of the VR2332 and JA142 viruses, the size of plaques produced by the viruses began to get bigger after 17 passages. However, overall plaque size still remained smaller than 2 mm in diameter, indicating that the cell growth phenotype (i.e., smaller-sized plaque) of wild-type PRRSVs is relatively stable during cell passages.

Genetic, antigenic, and phenotypic characteristics of PRRSV field isolates. Eighty-three field isolates were assayed for their susceptibilities to antisera raised against VR2332 or JA142. Then, the genetic relatedness of the field isolates to VR2332 and JA142 was assessed based on the ORF5 sequence. Six genotypic clusters were identified among the isolates examined. Twenty-five viruses (≥96.5% homology to VR2332/MLV) and 10 viruses (≥98.5% homology to JA142/ATP) were classified into clusters related to VR2332/MLV and JA142/ATP, respectively (Fig. 4). In the VN test, the infection of MARC-145 cells by 17 of the 25 VR2332/MLV-like virus and 9 of the 10 JA142/ATP-like viruses was significantly affected ($P < 0.05$) by VR2332 or JA142 antiserum, respectively (i.e., a less than fourfold decrease in the susceptibility of tested virus to antisera compared to that of the control viruses, VR2332 or JA142). In contrast, the infectivities of the remaining 48 viruses to MARC-145 were not significantly affected by the VR2332 or JA142 antiserum.

Thirteen of the 17 VR2332/MLV-like viruses and all 9 JA142/ATP-like viruses produced medium-sized (2- to 3-mm) to big-sized (>3-mm) plaques, which were similar to those produced by the MLV and ATP strains. Therefore, these 22 viruses were defined as VLVs, since they were antigenically and genetically close to VR2332/MLV or JA142/ATP and still maintained the phenotype of the vaccine virus (i.e., bigger-sized plaques). All 61 field isolates except one produced small-sized plaques (<2 mm), similar to those produced by VR2332 and JA142. Interestingly, the one virus designated 2M11715 was not closely related to either VR2332 or JA142 (90.9% or 90% ORF5 nucleotide homology to each virus) and produced medium-sized plaques (2.5 mm).

Relationship between phenotypic and genetic similarities of field isolates. As shown in Fig. 4 and 5, the genetic proximity of the ORF5 amino acid sequence of the viruses to VR2332 or MLV was not always well correlated with phenotypic characteristics. Five of the 12 viruses that were genetically close to MLV (99.5% homology) produced medium-sized (2- to 3-mm) or big-sized (>3-mm) plaques and the other seven viruses that were closely related to VR2332 (95 to 99.5% homology) produced small-sized plaques (<2 mm). However, the remaining 11 VR2332/MLV-like viruses had the same genetic distance

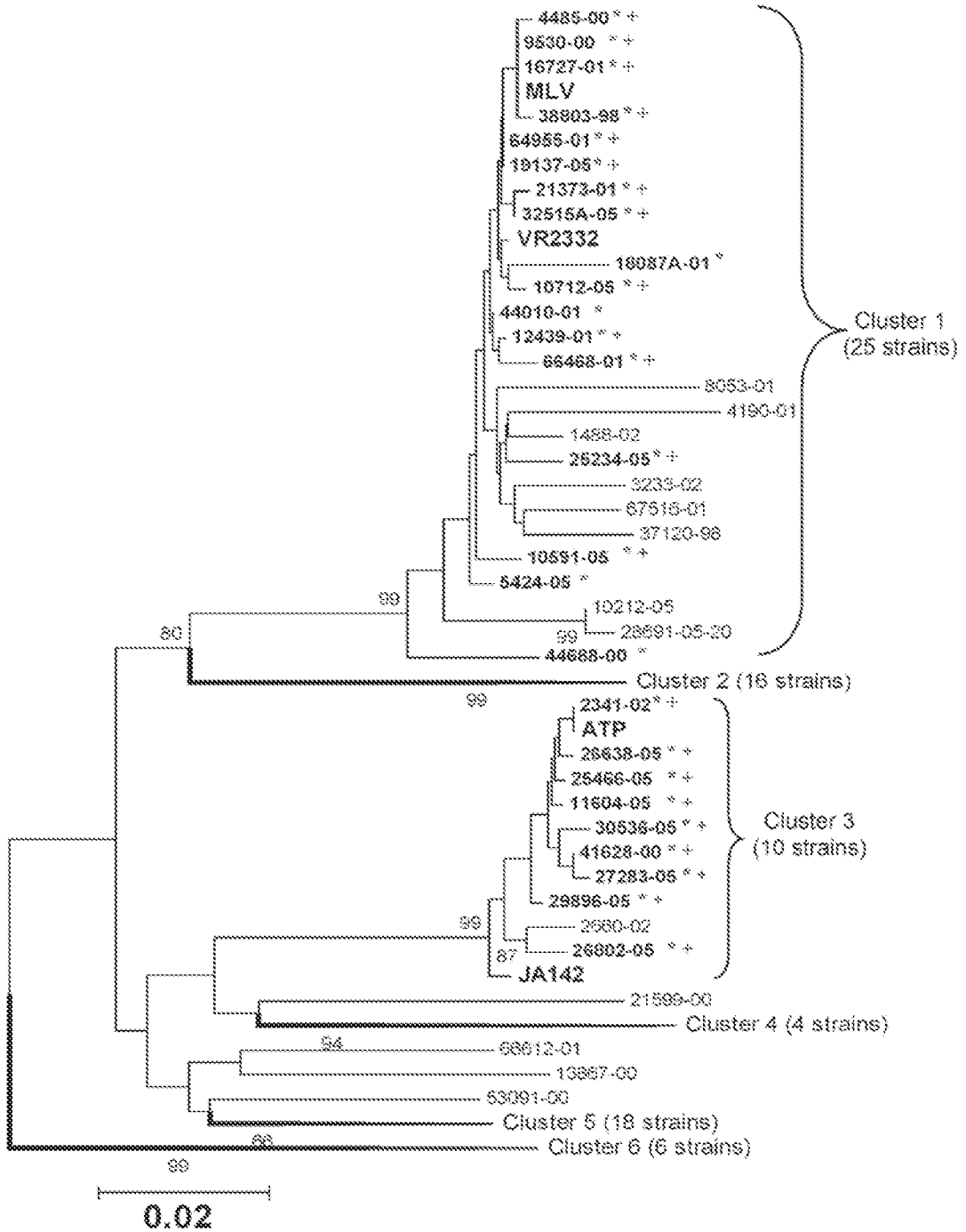

FIG. 4. Phylogenetic relationship of 83 PRRSV field isolates with the vaccine viruses (MLV, ATP) and their parental viruses (VR2332, JA142) based on ORF5 nucleotide sequence. Boldface and asterisks indicate isolates whose infections were significantly affected by VR2332 or JA142 antiserum. The symbol + indicates VLVs, which produced medium- to big-sized plaques. The reliability of analysis was determined by 1,000-times-repeated bootstraps.

from both VR2332 and MLV. Among those, six viruses (98.5 to 99.5% homology to both viruses) produced medium- or big-sized plaques, whereas the remaining five viruses (95% to 99% homology to both viruses) produced small-sized plaques. In addition, two viruses, which were genetically closer to VR2332 (98 and 99% homology, respectively), produced medium- or big-sized plaques.

In contrast, the genetic proximity of the ORF5 amino acid sequence of the viruses to JA142 or ATP appeared to have a better correlation with phenotypic characteristics. Seven viruses closely related to ATP (98.5 to 99.5% homology) produced a medium- or big-sized plaque, and one virus closely related to JA142 (97% homology) produced a small-sized plaque. Nonetheless, two viruses genetically closer to JA142

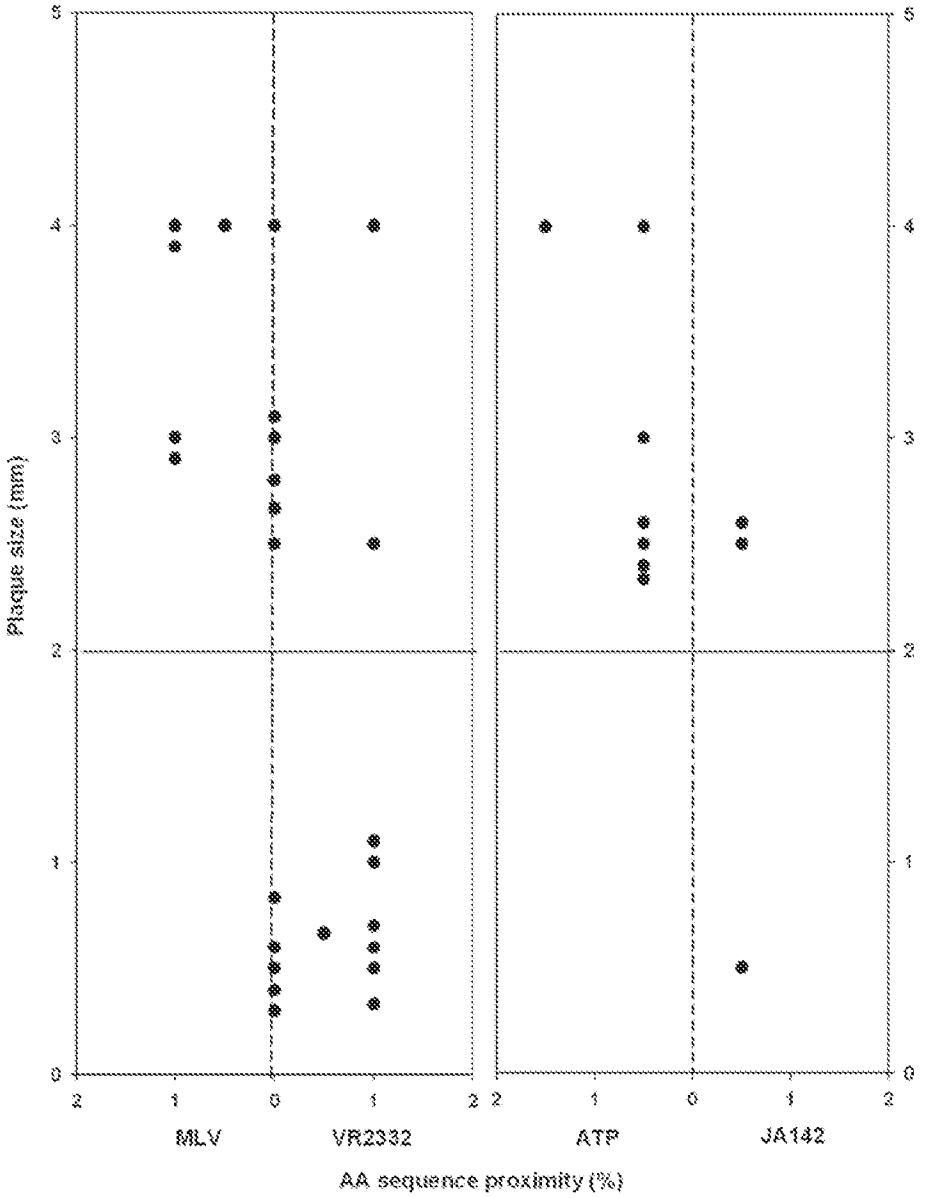

FIG. 5. The relationship in plaque size and sequence proximity of between 35 field isolates defined as VLVs and the vaccine strains (MLV and ATP) or their wild-type parental strains (VR2332 and JA142). The horizontal solid line at 2 mm of plaque size indicates the minimum size for medium-sized plaques. The vertical dotted lines at 0% indicate that the virus on the line is located at the identical genetic distance from both viruses. AA, amino acid.

based on ORF5 amino acid sequence (98% homology) produced a big-sized plaque, indicating that ORF5 sequence homology may not be able to differentiate VLVs from wild-type viruses.

Genetic markers for PRRSVs with vaccine virus-like phenotype. By comparing sequences of ORF2 to -7 among vaccine viruses, VLVs, and wild-type viruses, four unique amino acids were identified for each of the MLV and ATP vaccine strains (Tables 1 and 2). Phenylalanine at position 10 (F^{10}) in ORF2a and tyrosine at 9 (Y^{9}) in ORF2b, which arose from the same nucleic acid change, glycine at 151 (G^{151}) in ORF5, and glutamate at 16 (E^{16}) in ORF6 were found only in the MLV. Glutamate at 85 (E^{85}) and tyrosine at 165 (Y^{165}) in ORF3, serine at 80 (S^{80}) in ORF5, and cysteine at 62 (C^{62}) in ORF6 were found only in the ATP. Among these unique sequences, F^{10} in ORF2a and E^{85} and Y^{165} in ORF3 were the most stable and consistent sequence elements for MLV-like and ATP-like PRRSVs, respectively, since those amino acids were identified only in the vaccine viruses and VLVs that produced medium- to big-sized plaques among the 83 field isolates examined (Table 2).

Stability of unique amino acid sequences of vaccine viruses or VLVs during passages in animals and MARC-145. Amino acid sequences F^{10} in ORF2a and Y^{9} in ORF2b of the CC-01 strain (MLV-like virus) reverted to the sequences of VR2332, i.e., leucine (L) and aspartate (D), respectively, after three passages in the pigs (lines A and C). In line B, however, F^{10} was still observed in ORF2a without reversion to L even after

TABLE 1. Unique amino acid sequences for MLV-like viruses

ORF (aa[a] no.)	Plaque size[b] (classification)	No. of viruses	No. of viruses with indicated amino acid at ORF position		
			VR2332	MLV	Other sequences
ORF2a (10)			L	F[c]	S
	Big to medium (MLV-like)	13	2	**11**	
	Small (VR2332-like)	12	11		1
	Small (others)[d]	58	25		33
ORF2b (9)			D	Y	N or H
	Big to medium (MLV-like)	13	2	**7**	4 (H)
	Small (VR2332-like)	12	11		1 (N)
	Small (others)[d]	58	50		8 (N)
ORF5 (151)			R	G	K, I, or N
	Big to medium (MLV-like)	13	≈10	**3**	
	Small (VR2332-like)	12	11		1 (I)
	Small (others)[d]	58	52		6
ORF6 (16)			Q	E	
	Big to medium (MLV-like)	13	6	**7**	
	Small (VR2332-like)	12	12		
	Small (others)[d]	58	58		

[a] aa, amino acid.
[b] Big to medium is bigger than or equal to 2 mm in diameter and small is smaller than 2 mm in diameter (plaque size).
[c] The unique sequences found only in MLV are in boldface.
[d] One isolate (2M11715) not closely related to either VR2332 or JA142 but producing a medium sized-plaque was included in this group.

13 sequential pig-to-pig passages of the virus, suggesting that F^{10} in ORF2a may be stable during in vivo passages of the MLV strain. The Y^9 in ORF2b was, on the other hand, changed into histamine (H) instead of D after three passages, which remained unchanged until the end of 13 pig passages. The H^9 in ORF2b was found in field isolates which produced a medium- to big-sized plaque (Table 1) and thus was considered to be a marker amino acid alternative to Y^9.

Similarly, the amino acid sequence E^{16} in ORF6 was substituted with glutamine (Q) after three passages in the pigs. In line C, however, the E^{16} was substituted with G instead of Q, which remained without any further alteration until the completion of 13 pig-to-pig passages. Therefore, G^{16} in ORF6 would be an intermediate form between E and Q, similar to H^9 in ORF2b, even though such an amino acid was not found in ORF6 of any of the field isolates examined in this study. In contrast, the amino acid sequence G^{151} in ORF5 quickly reverted to that of the parental virus, i.e., arginine (R), after the first passage in the pigs.

None of the amino acid sequences unique to the vaccine viruses were found in progeny viruses produced during 20 sequential passages of VR2332 or JA142 in MARC-145. All descendant viruses had the same amino acid sequences as VR2332 or JA142 at the determined sites during the passages, suggesting that over 20 passages might be required to have vaccine-specific sequences at the identified positions.

Biological role of the identified genetic markers. The identified MLV genetic markers were incorporated into the

TABLE 2. Unique amino acid sequences for ATP-like viruses

ORF (aa[a] no.)	Plaque size[b] (classification)	No. of viruses	No. of viruses with indicated amino acid at ORF position		
			JA142	ATP	Other sequences
ORF3 (85)			D	E[c]	
	Big to medium (ATP-like)	9		**9**	
	Small (JA142-like)	1	1		
	Small (others)[d]	74	74		
ORF3 (165)			F	Y	L
	Big to medium (ATP-like)	9		**9**	
	Small (JA142-like)	1	1		
	Small (others)[d]	74	71		3
ORF5 (80)			G	S	
	Big to medium (ATP-like)	9	2	**7**	
	Small (JA142-like)	1	1		
	Small (others)[d]	74	74		
ORF6 (62)			F	C	
	Big to medium (ATP-like)	9	2	**7**	
	Small (JA142-like)	1	1		
	Small (others)[d]	74	74		

[a] aa, amino acid.
[b] Big to medium is bigger than or equal to 2 mm in diameter and small is smaller than 2 mm in diameter (plaque size).
[c] The unique sequences found only in ATP are in boldface.
[d] One isolate (2M11715) not closely related to either VR2332 or JA142 but producing a medium sized-plaque was included in this group.

TABLE 3. List of mutants and their mutation profiles

Category	Name of mutant	Target ORF(s)	Mutation
Single-site mutation	P2L10F	ORF2a and -2b	L-to-F mutation at position 10 of ORF2a and D-to-Y mutation at position 9 of ORF2b
	P5R151G	ORF5	R-to-G mutation at position 151 of ORF5
	P6Q16E	ORF6	Q-to-E mutation at position 16 of ORF6
Multiple-site mutation	P25	ORF2 and -5	Combination of P2L10F and P5R151G
	P26	ORF2 and -6	Combination of P2L10F and P6Q16E
	P56	ORF5 and -6	Combination of P5R151G and P6Q16E
	P256	ORF2, -5, and -6	Combination of P2L10F, P5R151G, and P6Q16E

VR2332 infectious clone (Table 3). To characterize the mutants, the multistep growth curve (Fig. 6A) and the size of plaques they produced (Fig. 6B) were determined with MARC-145 cells. All of the mutants appeared to grow better than VR2332E which was rescued from the original infectious clone. The effect of those identified sequences on the replication of the virus in MARC-145 cells was, however, accumulative. The growth of mutants with single-site changes (i.e., P2L10F, P5R151G, and P6G16E) was not significantly enhanced compared to that of VR2332E ($P = 0.07$). In contrast, mutants with changes in two sites (i.e., P25, P26, and P56) grew significantly better than did single-site-changed mutants ($P = 0.01$) and VR2332E ($P = 0.002$). Likewise, P256, i.e., a mutant with changes in three sites, grew significantly better than the mutants with changes in two sites ($P = 0.008$), although its growth was still less than that of MLV.

A similar observation was also made for the effect of the identified sequences on the size of plaques. The size of the plaques produced by the mutants became bigger and bigger when more of the identified genetic markers for MLV were incorporated into the VR2332 infectious clone (Fig. 6B).

DISCUSSION

A high level of genetic and antigenic variability that exists among PRRSVs is well documented. Therefore, genetic analysis tools such as sequencing and restriction fragment length polymorphism have been utilized to aid in better decision

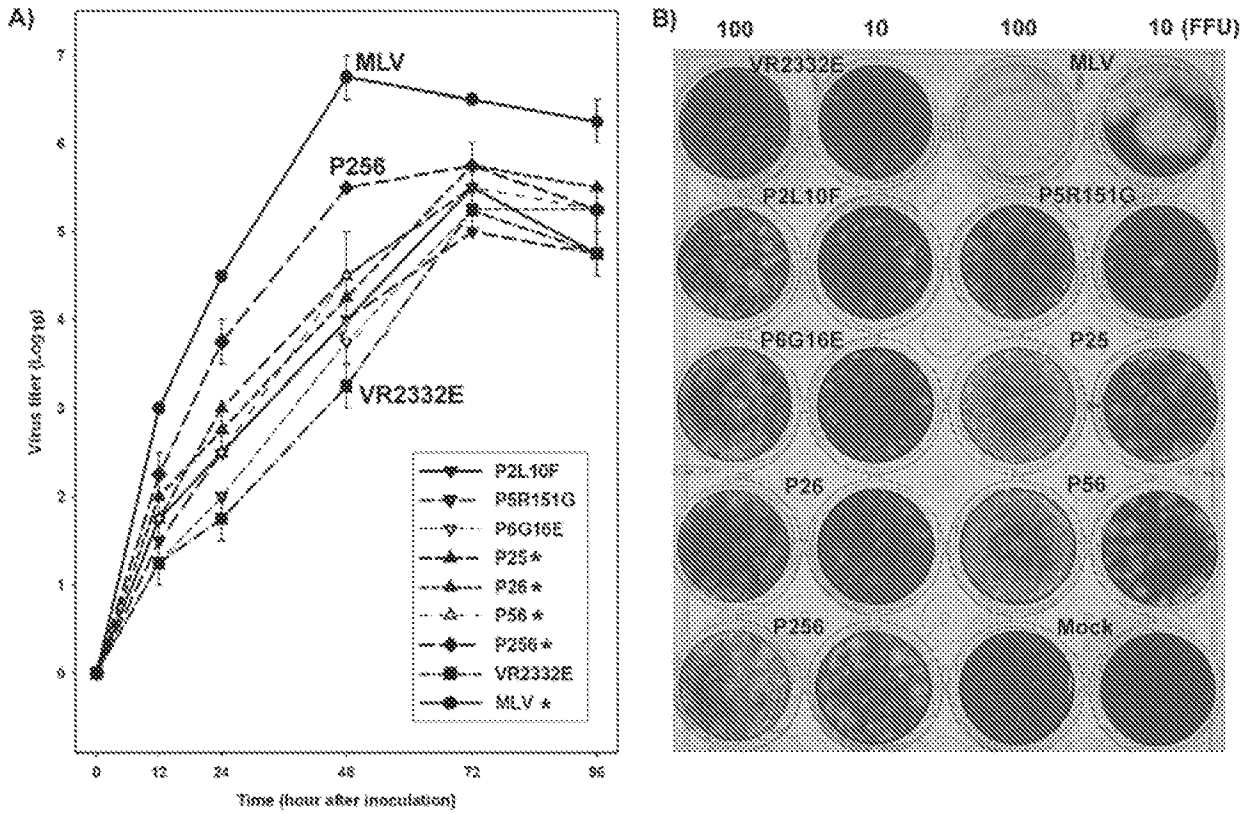

FIG. 6. Growth characteristics of a modified live PRRSV vaccine (MLV), the parental strain of the vaccine (VR2332E), and mutants constructed from a VR2332-based infectious cDNA clone with amino acid substitution(s) in one or more structural proteins of VR2332 toward MLV, as determined by multistep growth curve (A) and the size of plaque they produced (B) with MARC-145 cells. Asterisks in panel A indicate that the viruses grew significantly better than VR2332E, as determined by the multiple analysis of variation test. Numbers at the top of panel B indicate the amounts of virus inoculated to MARC-145 cells (duplicate wells/virus), as expressed in FFU per 0.1 ml.

making on the introduction of a new strain into the farm, the use of pig flow as a preventive measure, and intervention (i.e., commercial vaccine versus autogenous vaccine). Unfortunately, it has been common to detect field isolates which are genetically indistinguishable from both the vaccine virus and its wild-type parental strain because the vaccine strains are genetically very close to their parental strains. There is only a 0.3 or 1% difference in the sequence of ORF5, which is one of the most variable genes of PRRSV, between MLV and VR2332 or between ATP and JA142, respectively. MLV differs in full-length sequence from VR2332 by only 44 nucleotides (31). Considering that all commercial PRRS vaccines are modified live virus products without any marker, it is reasonable to expect that many of those indistinguishable field isolates may have been derived from the vaccine strains, since the vaccine virus is known to spread to unvaccinated animals and PRRSV is known to be continuously mutated during in vivo replication (26). However, the possibility that the parental strain of the vaccine virus, such as VR2332 or JA142, has been circulating in the pig population still exists. At present, no convenient way to reliably discriminate the vaccine viruses from their wild-type parental viruses is available.

In the present study, the vaccine viruses demonstrated highly efficient growth in the cell line MARC-145 (monkey kidney cell line), resulting in plaques of sizes significantly bigger than those seen for their wild-type parental strains. As the vaccine viruses are known to be attenuated through sequential passages in the cell line, the observed biotypic characteristic (i.e., better growth in the cells) was an expected outcome. It was interesting that such a difference of viral growth in MARC-145 was also observed for some of the field isolates, which provided the basis for identifying viruses derived from the vaccine strains in conjunction with sequence analysis. The plaque assay in combination with sequencing of ORF5 can be a very powerful tool for detecting VLVs, since it utilizes both genotypic and phenotypic characteristics of the virus. Several difficulties would be expected if the plaque assay alone is used for the purpose of virus differentiation. First, the plaque assay requires virus isolates, which is not always easy to get from clinical cases. Second, field isolates which are genetically as different from MLV as ATP (approximately 10% nucleotide difference) can produce a medium-sized plaque, as demonstrated in this study. Third, the phenotype of VLV can revert to that of a wild-type when the virus passed through animals, as evident in the present study (Fig. 3). These foreseeable difficulties necessitate the combined use of plaque assay and sequence comparison, so that the plaque assay can be performed on viruses which are indistinguishable from both vaccine and parental strains by gene sequencing (e.g., ORF5).

It was somewhat unexpected that a vaccine-like plaque phenotype of the virus (CC-01) completely reverted to the wild-type plaque phenotype (i.e., small-sized plaque) after three or four sequential passages in the pigs. Such faster reversion is believed to be attributed to the fact that the virus was not highly attenuated in cell culture like MLV vaccine viruses and was passed in pigs via an unnatural process (i.e., intramuscular injection of tissue homogenate containing the virus) (6). Vaccine viruses neither replicate well in pigs nor produce high levels of viremia after vaccination (10). Also, low levels of virus are detected from semen and respiratory tracts after vaccination compared to what is seen for wild-type viruses (5, 26). Therefore, the reversion of the vaccine phenotype to the wild-type phenotype during natural processes is expected to be slower than what was observed in the present study, since the vaccine virus likely needs considerable time to be adapted to the host and evolves slowly by maintaining a low level of replication rather than spreading through many pigs in a short time.

A noteworthy finding in the present study was the identification of amino acid sequences in ORF2, -3, -5, and -6, which were unique for the vaccine viruses or VLVs among the field isolates examined. Reverse genetics demonstrated their significant role in the plaque phenotype of the virus, suggesting that these sequence elements can be utilized as surrogate genetic markers for the vaccine viruses or VLVs. The identification of these surrogate markers for the vaccine viruses or VLVs should make it possible to determine genetic and phenotypic traits of a virus simply by gene sequencing without performing the laborious plaque assay. Among the sequence elements, G^{151} in ORF5 and E^{16} in ORF6 have been suggested as cell attenuation markers (21), implying that some or all of the identified markers could be related to PRRSV virulence. This postulate could be substantiated by the presence of VR2332 sequences at all of these identified sites in MLV-like viruses isolated from Danish pigs, which caused severe PRRS outbreaks after vaccination (16). Hence, the identified surrogate genetic markers also would provide a quick way to monitor the reversion of the vaccine virus to a wild type. Nonetheless, the role of nonstructural genes and their products in the virulence of PRRSV remains to be further studied.

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EXHIBIT C

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Generation of an Infectious Clone of VR-2332, a Highly Virulent North American-Type Isolate of Porcine Reproductive and Respiratory Syndrome Virus

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A full-length cDNA clone of the prototypical North American porcine reproductive and respiratory syndrome virus (PRRSV) isolate VR-2332 was assembled in the plasmid vector pOK_{12}. To rescue infectious virus, capped RNA was transcribed in vitro from the pOK_{12} clone and transfected into BHK-21C cells. The supernatant from transfected monolayers were serially passaged on Marc-145 cells and porcine pulmonary alveolar macrophages. Infectious PRRSV was recovered on Marc-145 cells as well as porcine pulmonary macrophages; thus, the cloned virus exhibited the same cell tropism as the parental VR-2332 strain. However, the cloned virus was clearly distinguishable from the parental VR-2332 strain by an engineered marker, a *BstZ*17I restriction site. The full-length cDNA clone had 11 nucleotide changes, 2 of which affected coding, compared to the parental VR-2332 strain. Additionally, the transcribed RNA had an extra G at the 5′ end. To examine whether these changes influenced viral replication, we examined the growth kinetics of the cloned virus in vitro. In Marc-145 cells, the growth kinetics of the cloned virus reflected those of the parental isolate, even though the titers of the cloned virus were consistently slightly lower. In experimentally infected 5.5-week-old pigs, the cloned virus produced blue discoloration of the ears, a classical clinical symptom of PRRSV. Also, the seroconversion kinetics of pigs infected with the cloned virus and VR-2332 were very similar. Hence, virus derived from the full-length cDNA clone appeared to recapitulate the biological properties of the highly virulent parental VR-2332 strain. This is the first report of an infectious cDNA clone based on American-type PRRSV. The availability of this cDNA clone will allow examination of the molecular mechanisms behind PRRSV virulence and attenuation, which might in turn allow the production of second-generation, genetically engineered PRRSV vaccines.

Porcine reproductive and respiratory syndrome is considered one of the most economically important infectious diseases of swine (1, 15). The disease is associated with severe reproductive disorders in sows and gilts and respiratory problems in pigs (11, 13, 16). The causative agent of the disease is porcine reproductive and respiratory syndrome virus (PRRSV), which, together with equine arteritis virus, lactate dehydrogenase-elevating virus, and simian hemorrhagic fever virus, are placed in the *Arteriviridae* family, within the order *Nidovirales* (9).

The PRRSV genome consists of a 5′-capped and 3′-polyadenylated single-stranded positive-sense RNA molecule of 15.1 to 15.5 kb. The viral genome contains, in the 5′ to 3′ direction, a 5′ leader, at least nine open reading frames (ORF 1a, ORF 1b, and ORFs 2 to 7), and a 3′ nontranslated region (14, 23, 32, 39). ORF 1a and ORF 1b are located directly downstream of the 5′ leader and encode a large replicase polyprotein, which is thought to be autoproteolytically cleaved into 13 smaller nonstructural proteins assumed to be involved in virus replication and transcription (4, 35, 38). ORFs 2 to 7 encode the structural proteins associated with the virion. These proteins are expressed from a 3′-coterminal nested set of subgenomic mRNAs (23, 24, 32).

PRRSV emerged almost simultaneously in North America and Europe in the late 1980s and early 1990s, respectively. Even though the same PRRSV-associated disease was observed on both continents, phylogenetic analysis has revealed two distinct genotypes of PRRSV, North American and European, with a sequence homology of only approximately 63% at the nucleotide level (2).

The establishment of infectious full-length cDNA clones has become critical in the study of viruses. The availability of such cDNA clones offers an opportunity for analysis and modification of viral genomes at the molecular level and has greatly aided research on virus replication, pathogenesis, and vaccine development (7). Yet, to date, only one infectious cDNA clone of PRRSV has been established, and this clone is unfortunately not generally available to the scientific community. Furthermore, this cDNA clone is based on the European-type Lelystad virus (25). The large genetic differences observed between the North American and European genotypes of PRRSV make it

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TABLE 1. Summary of oligonucleotides used for RT-PCR amplification

Primer[a]	Sequence (5′ → 3′)[b]	Position within VR-2332 genome[c]
5′ terminus (fragment b) primers		
RT416	5′ TGC GAT TGG A 3′	435–445
F20	5′ CTC GAG GGC GCG CC*T AAT ACG ACT CAC TAT AGG* TAT GAC GTA TAG GTG TTG GCT 3′ (*Xho*I, *Asc*I)	1–21
R229	5′ CGT GTG CAG TAT ACT TGG CCC T 3′ (BstZ17I)	249–270
Fragment e primers		
RT4890	5′ AAG GCT TGG A 3′	4910–4919
F251	5′ GGA GGG CCA AGT ATA CTG CAC ACG A 3′ (*Bst*Z17I)	247–271
R4774	5′ GTG TCA GGG TCA ACC ACG A 3′	4794–4812
Fragment f primers		
RT7924	5′ TGC ATC AGC A 3′	7944–7953
F4333	5′ ATC TTG GCT GGA GCT TAC GT 3′	4334–4353
R7821	5′ TGG TTG TGC TCA ACC GCG T 3′	7841–7859
Fragment d primers		
RT13019	5′ AGC TGA AGG A 3′	13039–13048
F7406	5′ TCT CAG AGT TGG CGA CCC T 3′	7408–7426
R12887	5′ ATC CTG CAC CAA AGA GAC CT 3′	12907–13026
Fragment c primers		
RT15327	5′ AAT TGA ATA GGT 3′	15347–15358
F12513	5′ TTT CAG CAT CTA GCC GCC A 3′	12515–12533
R15278	5′ AAT CAG TGC CGT TAA CCA CAC ATT CTT CCA 3′ (*Hpa*I)	15298–15327
3′ terminus (fragment a) primers		
RT15392	5′ CAG GAA ACA GCT ATG ACA CCT GAT CTC TAG A AA CGT T$(T)_{38}$3′ (*Xba*I, *Acl*I)	Poly (A) tail
F15301	5′ AGA ATG TGT GGT TAA CGG CA 3′ (*Hpa*I)	15302–15321
M13	5′ CAG GAA ACA GCT ATG AC 3′	M13 sequence

[a] Primer names are organized in groups. Prefixes: RT, reverse transcription primer; F, forward PCR primer; R, reverse PCR primer.
[b] The T7 RNA polymerase promoter sequence in primer F20 is shown in italics. Restriction sites introduced by PCR are underlined and specified in parentheses at the end of the sequence. Silent mutations within the viral sequence are shown in boldface. Fragments a through f correspond to the letters in Fig. 1.
[c] The nucleotide positions within the VR-2332 genome are based on GenBank accession numbers PRU87392 and AF094475 (26, 30).

highly relevant to obtain infectious cDNA clones representing both genetic types of PRRSV.

The present article describes the establishment of an infectious full-length cDNA clone of the North American-type isolate VR-2332. VR-2332 was chosen because it is the prototypical North American-type isolate, is well characterized, and has been shown to be highly virulent for sows and piglets (5, 10, 13). In addition, VR-2332 forms the basis of an attenuated live vaccine (3, 22), the complete genome sequences of both VR-2332 and the vaccine virus have been published (3, 26, 41), and both VR-2332 and the vaccine virus are freely available. Given the availability of complete genome sequences for the vaccine and VR-2332 strains, the infectious clone of VR-2332 should be immediately applicable for the identification of genetic attenuation and virulence determinants in the PRRSV genome, with a view to the development of second-generation, genetically engineered vaccines.

MATERIALS AND METHODS

Cells and virus. The virus used in the present study was a third cell culture passage of the highly virulent North American-type isolate VR-2332 (American Type Culture Collection).

Marc-145 cells were grown in Eagle's minimal essential medium (EMEM) with 5% fetal calf serum. BHK-21C cells were grown in EMEM with 10% fetal calf serum. Porcine pulmonary alveolar macrophages (PPAM) were obtained by lung lavage of specific-pathogen-free piglets free of PRRSV, as previously described (6). Prior to use, PPAM were resuspended in EMEM with 5% fetal calf serum. All cells were maintained at 37°C in a humidified 5% CO_2 atmosphere.

Marc-145 and PPAM are fully permissive for VR-2332 and were used to propagate and titrate the virus. To rescue virus from the infectious cDNA clone, cells had to be transfected with in vitro-transcribed RNA. Pulmonary macrophages are difficult to transfect, and preliminary experiments showed a slightly better transfection efficiency with BHK-21C cells than Marc-145 cells (not shown). Although BHK-21C cells cannot be infected by VR-2332, infectious virus was generated after transfection with viral RNA, similar to the observation for Lelystad virus (25). Therefore, BHK-21C cells were used to rescue infectious virus by transfection with in vitro-transcribed RNA.

RNA extraction and RT-PCR. Viral RNA was isolated by binding to silica particles in guanidine thiocyanate as previously described (28, 29) and used immediately for cDNA synthesis.

cDNA synthesis was performed with SuperScript II reverse transcriptase (RT) (Invitrogen A/S, Taastrup, Denmark) and specific RT primers (Table 1). A total of six fragments, covering the complete VR-2332 genome (two small fragments representing the viral termini and four large fragments representing the internal part of the genome), were subsequently PCR amplified with *PfuTurbo* DNA polymerase according to the manufacturer's protocol (Stratagene, Aarhus, Denmark). The cycling conditions were 94°C for 1 min, then 30 cycles of 92°C for 20 s, 58°C for 60 s, and 72°C for 6 min, followed by 25 cycles of 92°C for 20 s, 58°C for 60 s, and 72°C for 6 min with the time increased by 10 s per cycle, and finally 72°C for 10 min.

A fragment representing the 5′ end of the viral genome immediately preceded by a T7 RNA polymerase site was made by PCR with the F20 sense and R229 antisense primers (Table 1, fragment b). The F20 primer contained, in the 5′ to 3′ direction, an *Xho*I site, an *Asc*I site, the T7 promoter, and nucleotides 1 to 21 of the VR-2332 sequence (based on GenBank accession number AF094475) (30). The R229 primer introduced a silent mutation at nucleotide 259 (based on GenBank accession numbers PRU87392 and AF094475) (26, 30), leading to the creation of a *Bst*Z17I restriction site (Table 1, fragment b). A fragment representing the 3′ end of the viral genome was constructed by reverse transcription with primer RT15392, containing, in the 5′ to 3′ direction, an M13 primer site, an *Xba*I site, an *Acl*I site, and a T_{38} sequence (Table 1, fragment a). The reverse transcription reaction was followed by PCR with M13 antisense (Invitrogen A/S) and F15301 sense primers (Table 1, fragment a). The F15301 primer contained silent mutations at nucleotides 15314 and 15318, introducing an *Hpa*I site at the 5′ end of the fragment.

The four fragments covering the main internal part of the genome (ORFs 1 to

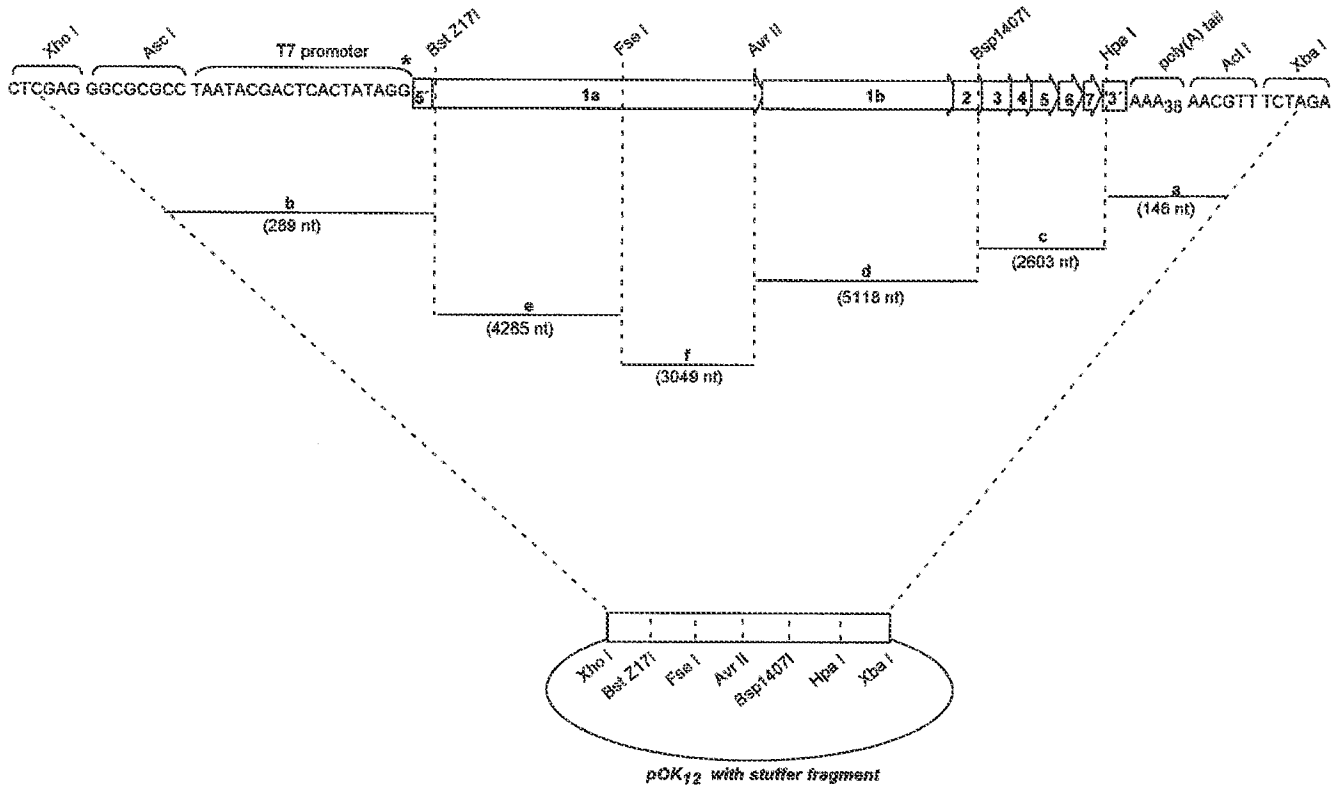

FIG. 1. Multistep strategy used to assemble full-length cDNA clone of VR-2332. In the top cartoon, the organization of the viral genome is shown, as are the positions of the unique restriction sites used for cloning purposes. The numbers 1a, 1b, and 2 through 7 indicate the PRRSV open reading frames. 5′ indicates the 5′ leader, and 3′ indicates the 3′ nontranslated region. At the 5′ end of the genome, *Xho*I and *Asc*I restriction sites and a T7 RNA promoter were fused to the genome. The asterisk indicates the transcription start site of T7 RNA polymerase, resulting in the sequence 5′-m^7G(5′)ppp(5′)G cap analog-TA TGA CGT ATA GGT. . .3′ as the predicted 5′ terminus of RNA transcribed in vitro with the T7 mMessage Machine kit. Downstream of the 3′ nontranslated region, a poly(A) tail of 38 A's and the restriction sites *Acl*I and *Xba*I were inserted. The complete viral genome was divided into six fragments flanked by unique restriction sites, represented by the horizontal lines labeled a through f. The length of each fragment is indicated in parentheses below the horizontal lines (in nucleotides). As shown in the bottom cartoon, these fragments were individually cloned into the pOK_{12} vector in the order indicated by the letters a to f. Prior to viral genome assembly, pOK_{12} was prepared by inserting a stuffer fragment containing all the unique restriction sites shown in the top cartoon in the *Xho*I and *Xba*I sites.

7, divided among fragments c through f; Fig. 1 and Table 1) were designed to allow assembly with unique restriction sites naturally found in the viral sequence. The primers used for RT-PCR amplification of these four internal fragments of the viral genome are described in Table 1 (fragments c through f). Finally, a small stuffer fragment which contained all these unique restriction sites (*Xho*I, *Bst*Z17I, *Fse*I, *Avr*II, *Bsp*1407I, *Hpa*I, and *Xba*I) was made by PCR with two overlapping synthetic oligonucleotides.

All PCR-amplified fragments were gel purified and cloned in the pCR-Blunt II-Topo vector (Invitrogen A/S). Before being used for assembly of the full-length clone (see below), these individual subclones were cycle sequenced with fluorescent BigDye chain terminators (Applied Biosystems, Nærum, Denmark), and the sequences were determined by capillary electrophoresis on an ABI Prism 310 genetic analyzer (Applied Biosystems).

Construction of full-length cDNA clone. A full-length cDNA clone of VR-2332 was assembled by following the multistep strategy illustrated in Fig. 1. First, the pOK_{12} plasmid (37) was prepared by inserting the above-mentioned stuffer fragment in the *Xho*I and *Xba*I sites. Then, each of the viral subclones (Fig. 1, fragments a through f) was excised from pCR-Blunt II-Topo, gel purified, and ligated into the pOK_{12} plasmid after digestion of pOK_{12} with the same restriction enzymes. Following each ligation step, the pOK_{12} construct was transformed into *Escherichia coli* DH5α cells and grown overnight at 37°C in the presence of kanamycin (50 μg/ml).

Thus, in order to assemble the total VR-2332 genome in pOK_{12}, six sequential rounds of cloning were performed in the following order. The fragment covering ORF7 and the 3′ nontranslated region, followed by the 5′ leader, ORFs 3 to 7, ORFs 1b and 2, and finally the two fragments of ORF 1a (Fig. 1; the lettering a through f indicates the order in which the viral subclones were inserted in pOK_{12}). To ensure that no deletions had occurred during the multiple clonings, several cDNA clones were tested by single digestion with the multicutting enzyme *Pvu*II or *Pst*I. Finally, after assembly was complete, the complete VR-2332 sequence in the pOK_{12} plasmid (complete 15.4-kb fragment flanked by *Xho*I and *Xba*I sites in Fig. 1) was resequenced, and the result was submitted to GenBank.

In vitro transcription and transfection. The full-length cDNA clone was linearized by cleavage with *Acl*I, which cuts downstream of the poly(A) tail. Linearized plasmid DNA was used for in vitro transcription of capped RNA [m^7G(5′)ppp(5′)G cap analog] with the mMessage Machine kit (Ambion) according to the manufacturer's instructions and including treatment of the RNA with DNase to remove input plasmid. The RNA was purified by acid phenol-chloroform followed by isopropanol precipitation and redissolved in Tris-EDTA buffer by heating at 70°C. To check the size and quality of the in vitro-transcribed RNA, a sample was denatured in urea-based RNA sample buffer (New England Biolabs, Hellerup, Denmark) and electrophoresed on a 1% native agarose gel in Tris-borate-EDTA buffer with 1 μg of ethidium bromide per ml.

For transfection, BHK-21C cells were seeded in six-well plates (200,000 cells/well in 2 ml of medium) and grown overnight to approximately 80% confluency. Then 5 μg of in vitro-transcribed RNA was mixed with 10 μl of DMRIE-C (1,2-dimyristyloxypropyl-3-dimethyl-hydroxy ethyl ammonium bromide and cholesterol) (Invitrogen A/S) and added directly to the medium. As a negative control, DMRIE-C without RNA was added to BHK-21C cells. As a positive control, BHK-21C cells were transfected with viral RNA from the parental VR-2332 isolate. This control RNA was extracted by acid phenol-chloroform purification followed by binding of the RNA to silica particles in guanidine thiocyanate (29). After 4 h of exposure to DMRIE-C and RNA, the monolayers were washed, and fresh medium was added. Supernatants from cells at 24 h posttransfection were serially passaged first on Marc-145 cells (four passages, each for 5 days) and then on PPAM (one passage, 3 days).

Titration and detection of virus were performed with an immunoperoxidase monolayer assay essentially as described by Bøtner et al., with the monoclonal antibody SDOW17 directed against the PRRSV nucleocapsid protein (27).

Discrimination between the cloned virus and VR-2332. Viral RNA was extracted with the QiaAmp viral RNA minikit (Qiagen, Hilden, Germany) and reverse transcription with Ready-to-go RT beads (Amersham Bioscience, Hørsholm, Denmark) and random hexamers was performed as described previously (29). A 990-bp fragment containing the *Bst*Z17I site engineered in the cloned virus was PCR amplified with the primers 5′-GCA TTT GTA TTG TCA GGA GCT-3′ and 5′-CAA GTC AAA CAA GCT CCA CC-3′. RT-PCR amplicons were digested with *Bst*Z17I and analyzed on a 2.5% agarose gel.

Inoculation in pigs. Twelve 5.5-week-old pigs from a specific-pathogen-free and PRRSV-seronegative herd were divided into three groups, each consisting of four animals. The pigs were Landrace/Yorkshire crossbred. The first group received $10^{4.8}$ 50% tissue culture infectious doses ($TCID_{50}$) of the cloned virus (fourth passage on Marc-145 cells) per ml, the second group received $10^{5.4}$ $TCID_{50}$ of the parental virus isolate VR-2332 (fourth passage on Marc-145 cells) per ml, and the third group was mock inoculated with EMEM. All the animals received 1 ml of inoculum in each nostril. The animals were kept in separate rooms throughout the experiment and observed daily for clinical signs of disease. Blood samples were collected on days 0, 7, 14, and 21 postinfection and tested for PRRSV-specific antibodies by blocking enzyme-linked immunosorbent assay and the immunoperoxidase monolayer assay (6, 33). All pigs were euthanized on day 21 postinfection.

Nucleotide sequence accession number. The complete genomic sequence for the infectious full-length cDNA clone of VR-2332 described in this report has been deposited as GenBank accession number AY150564.

TABLE 2. Nucleotide differences between the parental VR-2332 isolate and the full-length cDNA clone

Nucleotide position within VR-2332 genome[a]	"Consensus" nucleotide of North American isolates[b]	Nucleotide in cDNA clone	Change
259	G	A	Silent
1075	C	T	Silent
5520	C	T	Y → I
5611	T	A	Silent
6854	G	A	D → N
6967	T	C	Silent
7555	T	C	Silent
10644	T	C	Silent
13788	T	C	Silent
15314	G	T	Silent
15318	T	C	Silent

[a] Nucleotide positions within the VR-2332 genome are based on GenBank accession numbers AF094475 and PRU87392 (26,30).

[b] After final assembly in pOK_{12}, the infectious clone (15.4-kb fragment flanked by *Xho*I and *Xba*I sites in pOK_{12}; Fig. 1) was sequenced in total (GenBank accession number AY150564). This sequence was compared to a "consensus" sequence of previously published full-length sequences of North American isolates (GenBank accession numbers PRU87392, AF066183, AF176348, AF046869, and AF159149) (2,3,26,38,41). In total, 11 nucleotide differences were observed, as shown in the table. The mutations at nucleotide positions 259, 15314, and 15318 were introduced intentionally to create *Bst*Z17I and *Hpa*I restriction sites, respectively. The sites were used for cloning purposes (see Fig. 1). Furthermore, the *Bst*Z17I site was used as a genetic marker for the cloned virus.

RESULTS

Assembly of full-length cDNA clone of VR-2332. Marc-145 cells were infected with the VR-2332 isolate (third passage on Marc-145 cells) at a multiplicity of infection of 1.3 $TCID_{50}$/cell, and virus was harvested 5 days postinfection. From this material, a full-length cDNA clone covering the entire genome of the pathogenic North American isolate VR-2332 was assembled from overlapping PCR fragments flanked by unique restriction sites (Fig. 1). In order to minimize the number of PCR mutations, the amplifications were performed with a proofreading thermostable DNA polymerase (12). Nevertheless, even a proofreading polymerase would be expected to introduce mutations in a target of this size (Fig. 1; the North American-type PRRSV genome is 15.4 kb long). Therefore, to minimize PCR artifacts, the individual PCR fragments (labeled a through f in Fig. 1) were verified by sequencing before being selected for the final assembly. Finally, to ensure that the infectious clone was as well characterized as possible, the completely assembled VR-2332 sequence in the full-length clone (complete 15.4-kb construct flanked by *Xho*I and *Xba*I sites in pOK_{12}, as shown in Fig. 1) was DNA sequenced.

In total, 11 nucleotide differences were identified (Table 2) when the DNA sequence of the full-length cDNA clone was compared to previously published full-length sequences of North American isolates (GenBank accession numbers AF046869, AF066183, AF159149, AF176348, and PRU87392) (2, 3, 26, 38, 41). Three of these differences were silent mutations that were introduced intentionally to generate the *Bst*Z17I and *Hpa*I restriction sites (Tables 1 and 2). The remaining eight nucleotide differences were either PCR artifacts or the result of genetic variation in the VR-2332 isolate. Only two of the nucleotide mutations resulted in amino acid changes. These were at nucleotides 5520 (Tyr → Ile) and 6854 (Asp → Asn), both located in ORF 1a (Table 2).

Capped RNA was in vitro transcribed from the *Acl*I-linearized full-length cDNA clone with T7 RNA polymerase, and the quality of the RNA was verified by gel electrophoresis (data not shown). At the 5′ end, the in vitro-transcribed RNA had a nonviral G corresponding to the transcription initiation site of the T7 RNA polymerase (Fig. 1).

To recover infectious virus from the full-length cDNA clone, BHK-21C cells were transfected with the capped RNA with the transfection reagent DMRIE-C. Supernatants from the transfected BHK-21C cells obtained 24 h posttransfection were serially passaged four times on Marc-145 cells. Positive staining for nucleocapsid protein was detected in Marc-145 cells inoculated with supernatant from passage 1 (Fig. 2). As nucleocapsid production is a late event in PRRSV replication and requires the production of subgenomic mRNA, nucleocapsid staining strongly indicated that the infectious clone was not grossly impeded in any step of intracellular replication (Fig. 2). Similar results were observed after transfection with viral RNA from the VR-2332 isolate. However, no obvious cytopathic effect was detected earlier than passage 3 after transfection with the in vitro-transcribed RNA. This observation deviated from the results obtained when BHK-21C cells were transfected with viral RNA from the parental VR-2332 strain. In this case, cytopathic effect was observed as early as passage 1 on Marc-145 cells.

Finally, the supernatant from the fourth Marc-145 cell passage was passaged once on PPAM. Positive antinucleocapsid staining in the immunoperoxidase monolayer assay confirmed the presence of virus replication in PPAM. These results indicated that the cloned virus possessed the ability to replicate not only in Marc-145 cells but also in PPAM, as observed for the parental VR-2332 virus isolate.

Discrimination between cloned virus and VR-2332. To exclude that the results obtained from the cloned virus were artifactual, for example, due to laboratory contamination with the parental VR-2332 isolate, we assayed the cloned virus

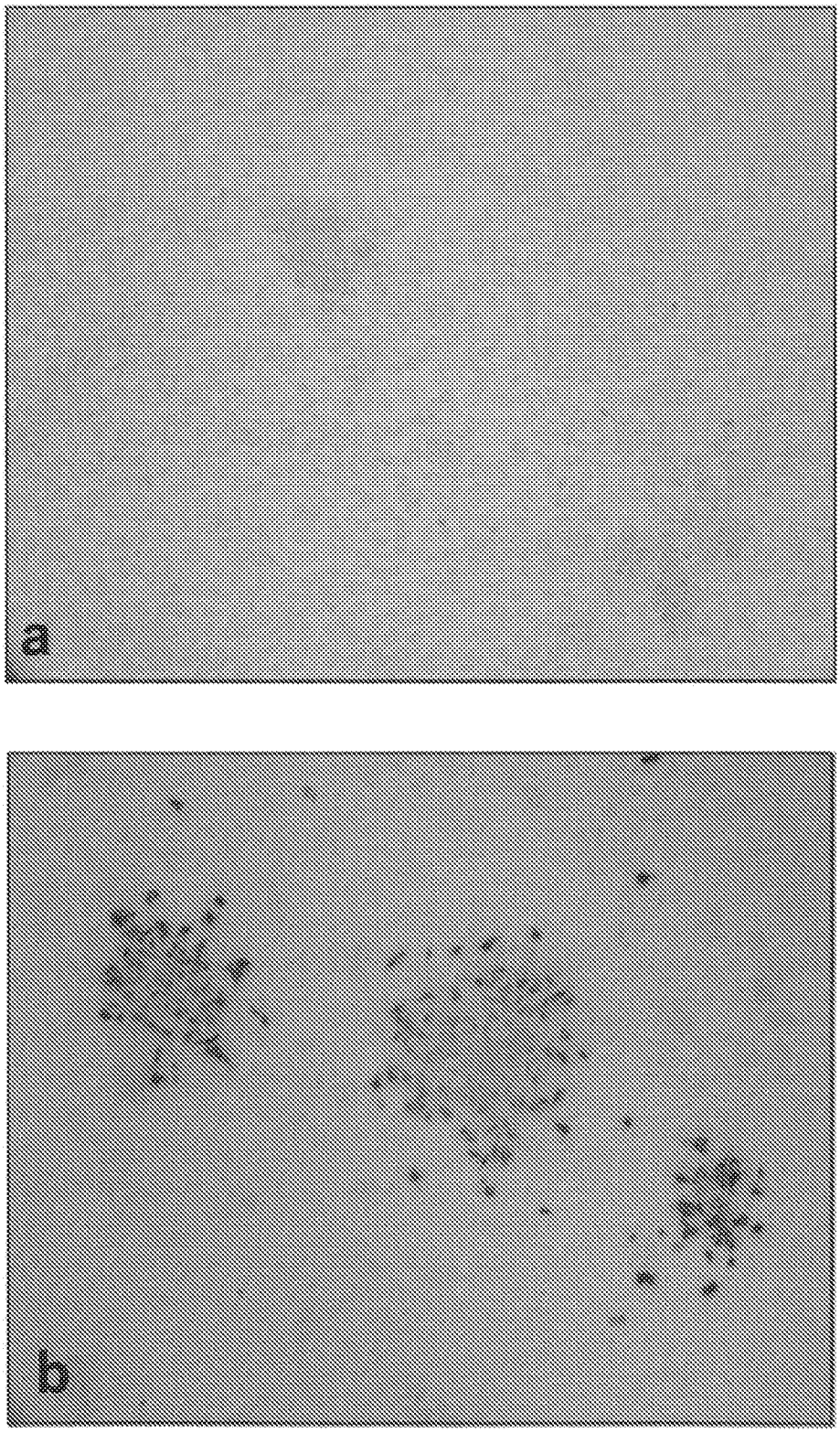

FIG. 2. Detection of cloned virus replication in Marc-145 cells. Supernatants from BHK-21C cells transfected with either (a) transfection reagent DMRIE-C without RNA as a negative control reaction or (b) RNA transcribed in vitro from the full-length cDNA clone were used to infect Marc-145 cultures. At day 3 after infection, the Marc-145 cultures were ethanol fixed and stained with monoclonal antibody SDOW17, directed against PRRSV nucleocapsid protein (a late marker of viral replication), and a horseradish peroxidase-conjugated secondary antibody (immunoperoxidase monolayer assay).

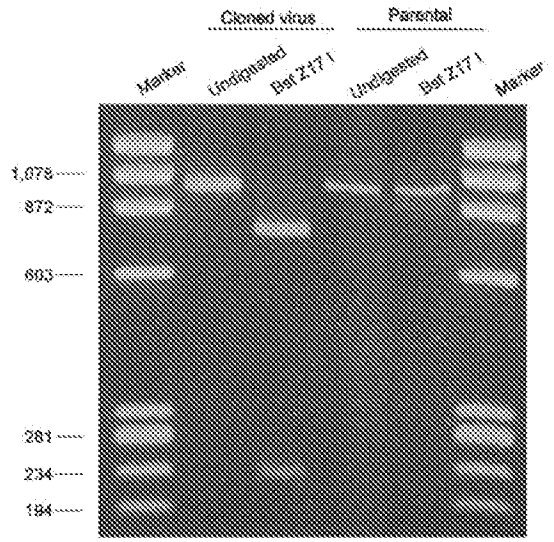

FIG. 3. Differentiation between cloned virus and parental VR-2332 strain. A *Bst*Z17I restriction site was introduced in the full-length cDNA clone of VR-2332 to allow discrimination between cloned virus (tagged with the *Bst*Z17I site) and parental virus (lacks a *Bst*Z17I site). RNA was extracted from lysates of cells infected with either the cloned virus or the parental VR-2332 isolate, and a 990-bp fragment was amplified by RT-PCR as described in Materials and Methods. The amplicons were digested with *Bst*Z17I and analyzed on a 2.5% agarose gel. The presence of a *Bst*Z17I restriction site resulted in fragments of 762 bp and 228 bp. As expected, the restriction site was found in the cloned virus but not in the parental VR-2332 virus isolate.

(second passage on Marc-145 cells) for the presence of a *Bst*Z17I restriction site. As expected, the RT-PCR fragment derived from the cloned virus was cleaved by *Bst*Z17I, generating two fragments of 228 bp and 762 bp (Fig. 3). In contrast, the PCR fragment derived from the parental isolate was not cleaved by *Bst*Z17I (Fig. 3).

Growth kinetics. To determine the growth curve of the cloned virus and to compare it to the growth curve of the parental VR-2332 strain, Marc-145 cells were infected with each of the viruses (fourth passage on Marc-145 cells) at a multiplicity of infection of 0.002 $TCID_{50}$/cell. After 2 h of incubation, the cells were washed twice, and fresh medium was added (time 0). The virus titers of the supernatants were determined by immunoperoxidase monolayer assay on Marc-145 cells at 0, 3, 16, 20, 24, 48, and 72 h postinfection.

The growth curves for the cloned virus revealed a first peak of replication at 16 h postinfection and a second peak at 48 h postinfection (Fig. 4). The same biphasic growth curve was obtained for the parental VR-2332 isolate (Fig. 4). The biphasic kinetics may represent a first and a second replication cycle of PRRSV in Marc-145 cells. Thus, the cloned virus displayed growth kinetics very similar to those of the parental VR-2332 isolate.

The titers of the parental virus were consistently slightly higher than the titers obtained for the cloned virus. This might indicate that the extra G at the 5′ end (Fig. 1) and the other nucleotide changes incurred during cloning (see above, Table 2) affected the replication of the cloned virus. However, the differences in titer between the cloned virus and VR-2332 were quite small at all times.

Inoculation in pigs. In order to investigate the infectivity of the cloned virus in vivo and to compare it to the infectivity of the parental VR-2332 isolate, we performed an inoculation experiment in young pigs. As VR-2332 is a highly virulent isolate, we could gauge infectivity by clinical signs. Additionally, the seroconversion kinetics were monitored.

A blue discoloration of the ears was detected at day 9 postinfection in two of four pigs inoculated with the cloned virus (Fig. 5). Also at day 9, four of four pigs inoculated with the parental VR-2332 isolate exhibited a blue discoloration of the ears (Fig. 5). This discoloration was transient, lasting approximately 6 days. All four pigs in the VR-2332 group exhibited inappetence and appeared lethargic on days 7, 8, and 9 postinfection. Furthermore, two of four pigs in the VR-2332 group exhibited lameness from day 10 postinfection. Postmortem results revealed hemopurulent infection of the knee joint in one pig and the elbow joint in the other. These findings indicated that the lameness was most likely caused by a bacterial infection, in agreement with the known predisposition of PRRSV-infected animals to secondary bacterial infections. Both these pigs appeared lethargic throughout the experiment. The four negative control pigs remained vigorous, had good appetites, and did not at any time show clinical signs of disease.

All pigs were seronegative prior to infection (day 0). In the group infected with the cloned virus, three of four pigs had seroconverted on day 7 postinfection and four of four pigs on day 14 postinfection (Table 3). In the VR-2332-infected group, all four pigs had seroconverted on day 7 postinfection (Table 3). PRRSV-specific antibodies were not detected in pigs from the negative control group (Table 3).

DISCUSSION

In the current work, we established an infectious cDNA clone of the highly virulent, prototypical North American PRRSV isolate VR-2332 (5, 10, 13). While the construction of infectious viral clones is becoming commonplace, the technical

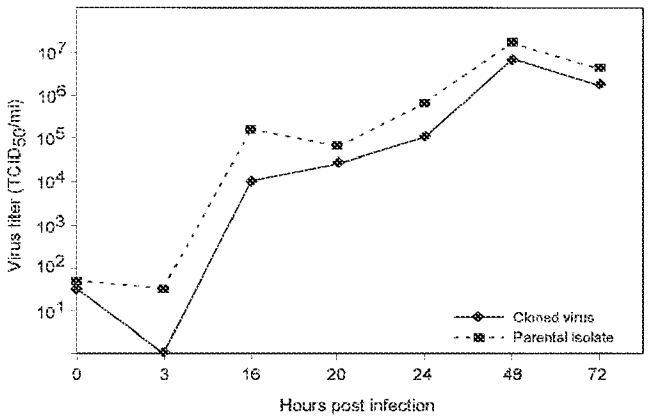

FIG. 4. Growth curves of cloned virus and parental VR-2332 isolate. Parallel cultures of Marc-145 cells were infected at a multiplicity of infection of 0.002 $TCID_{50}$/cell with virus recovered from the full-length cDNA clone and the parental VR-2332 isolate. After 2 h of incubation at 37°C, the cells were washed twice and fresh medium was added (time 0), and the cells were incubated at 37°C. At 0, 3, 16, 20, 24, 48, and 72 h postinfection, samples of the supernatants were taken, and virus titers were determined as described in Materials and Methods.

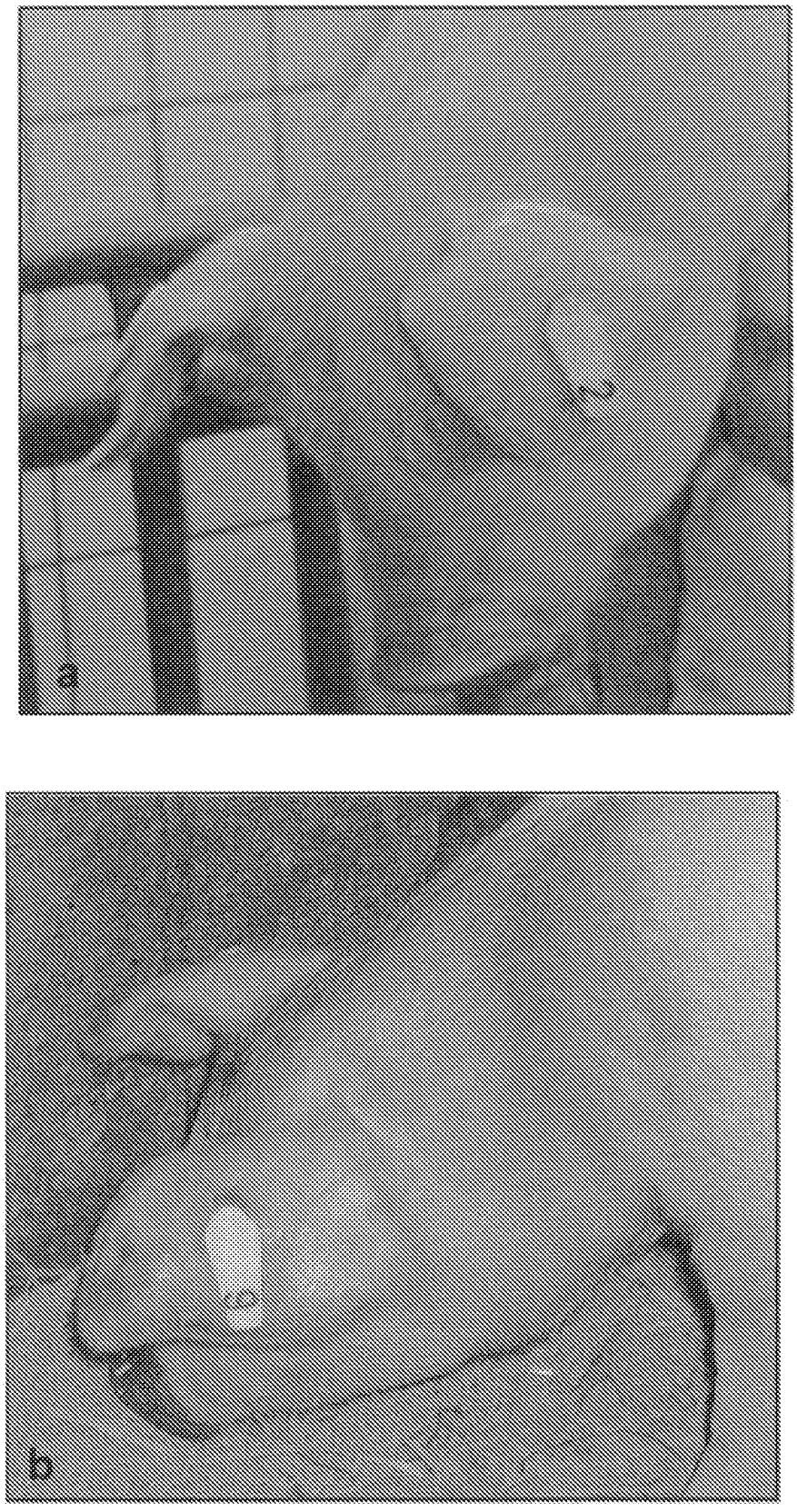

FIG. 5. Development of blue ears in pigs inoculated with cloned virus. A classical clinical sign of PRRSV infection, blue discoloration of the ears, was detected at day 9 postinfection in pigs experimentally infected with (a) the cloned virus or (b) the parental VR-2332 strain.

TABLE 3. Detection of PRRSV-specific antibodies in experimentally infected pigs[a]

Infection	Pig no.	Seroconversion (ELISA/IPMA result) on day postinfection:			
		0	7	14	21
Cloned virus	1	−/0	−/0	+/1,250	+/6,250
	2	−/0	+/50	+/1,250	+/6,250
	3	−/0	+/50	+/250	+/6,250
	4	−/0	+/0	+/6,250	+/6,250
Parental virus	5	−/0	+/250	+/6,250	+/6,250
	6	−/0	+/0	+/1,250	+/6,250
	7	−/0	+/0	+/1,250	+/6,250
	8	−/0	+/0	+/1,250	+/6,250
None (negative control)	9	−/0	−/0	−/0	−/0
	10	−/0	−/0	−/0	−/0
	11	−/0	−/0	−/0	−/0
	12	−/0	−/0	−/0	−/0

[a] Seroconversion was assayed by blocking ELISA and immunoperoxidase monolayer assay (IPMA), two tests routinely used for large-scale examination of field serum samples at the Danish Veterinary Institute. ELISA results: −, no anti-PRRSV antibodies detected; +, anti-PRRSV antibodies detected. Immunoperoxidase assay: sera were tested at 1:50, 1:250, 1:1,250, and 1:6,250 dilutions; 0, negative (anti-PRRSV antibodies not detected); positive results are indicated as the reciprocal of the highest serum dilution at which anti-PRRSV antibodies could be detected.

challenge involved in cloning the genomes of mammalian viruses in bacterial plasmids remains significant (7, 18, 19, 40). In particular, it is impossible to predict in advance how stable a given infectious clone will be during propagation in *E. coli*. Plasmid toxicity is not completely understood but may result from vector-specific as well as insert-specific causes (7, 18, 19, 40). Thus, a key parameter in infectious clone construction is the plasmid backbone. We used the low-copy pOK_{12} plasmid (37), which has been used previously for the assembly of an infectious clone of European-type PRRSV (25), and found that pOK_{12} also provided an appropriate backbone for an infectious clone of American-type PRRSV. Given the relatively large level of genetic differences between European-type and American-type PRRSV (2, 21, 26), we consider this a nontrivial technical achievement which paves the way for further infectious clones of North American-type as well as European-type PRRSV, for example, based on isolates differing in virulence.

The main design feature of our infectious clone is the division of the viral genome into six cassettes flanked by unique restriction sites, making future manipulation of the cloned virus very easy (Fig. 1, cassettes labeled a through f). Furthermore, the clone was completely sequenced (GenBank accession number AY150564), i.e., it has been fully genetically characterized. Finally, the clone is based on a highly virulent PRRSV strain which produces pronounced clinical and histopathological changes that are reproducible and easy to monitor during experimental infection of pigs. We expect that these features will make the infectious clone useful for the rapid identification of attenuation and virulence determinants in the PRRSV genome.

To rescue infectious virus from the pOK_{12} clone, full-length PRRSV genomic RNA was generated by in vitro transcription and transfected into BHK-21C cells. Since previous results indicated that a cap structure enhances the specific activity of transfected PRRSV genomic RNA (25), the cap analog $m^7G(5')ppp(5')G$ was included during in vitro transcription. By passaging supernatants from transfected BHK-21C cells on Marc-145 cells, stocks of infectious virus at a reasonable titer ($10^{4.8}$ $TCID_{50}$/ml, sufficient for experimental infections) could be obtained. Identification of a marker *Bst*Z17I site introduced in the cDNA clone confirmed that the recovered virus did not represent contamination by the parental VR-2332 strain.

An infectious clone should ideally be genetically completely identical to the parental virus. In practice, this is difficult to achieve. First, the sequence for the parental virus, especially when dealing with RNA viruses, may be ambiguous due to the heterogeneity (sometimes referred to as quasispecies structure) often seen in RNA viruses (20, 31). Second, during clone construction, some mutations will invariably occur during RT-PCR amplification (8, 12), other mutations have to be introduced for cloning purposes, and viral 5′ termini are sometimes difficult to reproduce faithfully with the T7 RNA polymerase system. Thus, comparing the biological properties of an infectious clone-derived virus and its parental strain is important. We found that, compared to the parental VR-2332 strain, the cloned virus exhibited the same cell tropism (replicated in Marc-145 cells as well as PPAM), exhibited essentially the same growth kinetics in Marc-145 cells (Fig. 4), and induced seroconversion in experimentally infected pigs (Table 3). Most importantly, experimental infection with the cloned virus induced a classical clinical sign of PRRSV infection, blue discoloration of the ears, which was also observed following infection with the parental VR-2332 strain. Thus, the cloned virus was qualitatively very similar to the parental VR-2332 strain. However, some quantitative differences were observed. First, the cloned virus replicated to slightly lower titers in Marc-145 cells (Fig. 4). Second, the seroconversion kinetics in pigs were marginally slower for the cloned virus (Table 3).

The quantitative biological differences between the cloned virus and VR-2332 mentioned above were minor and have as yet not been confirmed in repeated experiments. Thus, the slightly lower titers in Marc-145 cells (Fig. 4) might be due to experimental variability, and differences in the virus dose used for the animal experiments could account for the marginally quicker seroconversion kinetics (Table 3) and more pronounced clinical changes (see results) in the VR-2332-infected group. Alternatively, the quantitative biological differences observed between the cloned virus and VR-2332 might be real and reflect mutations and genetic defects in the cloned virus, which potentially include (i) 11 nucleotide mutations (Table 2), (ii) an extra 5′ nonviral G, derived from the T7 promoter (Fig. 1 and Table 1), and (iii) a relatively short poly(A) tail (Fig. 1 and Table 1).

The 11 mutations in the cloned virus might potentially influence replication. Only two of these mutations affected coding (Table 2). Both amino acid changes are conservative (17). However, the function of the ORF 1a protein segment, where both these coding mutations are located, is currently unknown, and it cannot be ruled out that they affect viral replication. In a similar vein, noncoding mutations might affect replication. For example, to obtain the fragment covering the 3′ end of the viral genome (Fig. 1, fragment a), an *Hpa*I restriction site was created by introduction of two silent mutations in the 3′non-

translated region (Table 1, primer F15301). With the infectious cDNA clone of the European strain Lelystad, Verheije et al. (37) showed that a highly conserved region in the 3′ nontranslated region folds into a stem-loop, which is engaged in a seven-nucleotide kissing interaction with a similar domain in ORF 7. These domains, which are highly conserved among PRRSV isolates, may play a critical role in virus replication. The mutations introduced by primer F15301 (Table 1) are both situated in this 3′ nontranslated region stem-loop (36). Even though none of the mutations are situated within the seven-nucleotide sequence essential for the kissing interaction, mutations anywhere in this domain might weaken the interaction, thereby reducing viral replication. Experiments are planned to evaluate whether the mutations introduced by primer F15301 (Table 1) interfere with virus replication.

Finally, it should be mentioned here that extension of the 5′ terminus is considered to have a substantial effect on virus replication (7). However, the presence of an additional 5′ nonviral G in the infectious cDNA clone of Lelystad virus did not seem to impair its growth (25). Furthermore, 5′ rapid amplification of cDNA ends of the European-type PRRSV isolate 111/92 revealed a further extension of two nucleotides at the 5′ end compared to the published Lelystad virus sequence (23, 30). Thus, it is possible that there is some natural heterogeneity in the 5′ end of the PRRSV genome without this having a major effect on viral replication.

As mentioned, there are substantial genetic differences between American-type and European-type PRRSVs. Additionally, the two virus types are antigenically very different, and there is only limited cross protection against reinfection with the heterologous type (20). That is, a genetically engineered vaccine based on, for example, an infectious clone of Lelystad virus would not be expected to offer full protection against infection with American-type PRRSV. Thus, the infectious clone of American-type PRRSV generated in the present study represents a significant new opportunity for recombinant vaccine development. Furthermore, previous studies on PRRSV have mainly focused on the functional role of the structural proteins. Regarding the nonstructural proteins, important information concerning their individual roles remains to be addressed. The availability of an infectious clone of VR-2332, supplementing the infectious clones of Lelystad virus (25) and the related equine arteritis virus (34), may broaden the questions that can be examined experimentally in the field of *Arterivirus* replication and pathogenesis.

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